Microbiological and physico-chemical assessment of surface water processed with low-cost and sustainable technology

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By

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List of Abbreviations

(+)ve	Positive
(-)ve	Negative
ATCC	American Type Culture Collection
et al	and others
etc.	et cetra
Fig.	Figure
hr.	Hour
kbp	Kilobase pair
bp	Base Pair
M	Molar
g	Gram
µg	Microgram
mg.	Miligram
L	Liter
mL.	Mililitre
µL	Microliter
nm	Nanometer
mm	Milimeter
cm	Centimeter
min	Minute
rpm	Rotation per minute
RT	Room temperature
vol.	Volume
w/v	Weight per volume
%	Percentage
°C	Degree Celsius
pH	Negative logarithm of hydrogen ion concentration
VBNC	Viable but non-culturable
sec.	Second
sp.	Species (singular)
spp.	Species (plural)
PCR	Polymerase Chain Reaction
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylene Diamine Tetra-Acetic Acid
TAE	Tris-Acetate EDTA
E. coli	*Escherichia coli*

Abstract

Surface water has been used for drinking purpose from ancient times. However, with increasing population and human activity this water became contaminated with pathogens, disease vectors or unacceptable levels of toxins or suspended solids. In Bangladesh, tube wells came as a solution, but soon it turned out to be a menace as it was contaminated with arsenic in many areas. Cities and towns are supplied with expensive large scale water treatment plants but this is not viable for the majority of the population in Bangladesh who live in isolated villages. A similar situation exists in most of the low resource countries of the world. Therefore, small domestic scale solutions for drinking water are needed for villages which are low-cost, simple technology and easy to maintain.

Surface water is free of arsenic since it comes mostly from rain and melted snow. As this water seeps through different layers of the ground it picks up different minerals to become ground water aquifers; poisonous arsenic will also be present in the intermediate rocks. Since arsenic is difficult to remove in a domestic scale arrangement while destroying enteropathogenic microorganisms is simpler. So, use of arsenic free surface water treated using different simple techniques could be more desirable for the rural areas of the low resource countries like Bangladesh.

From the perspective of human consumption, surface water is contaminated with enteropathogenic microorganisms, including those of diarrhea, cholera, typhoid, paratyphoid, jaundice etc., which need to be removed to make the water safe for drinking. So, the present study was basically taken up with the aim to study and evaluate the success of three new technologies that can disinfect surface water from diarrhoeal disease causing microorganisms. First one is low-cost solar pasteurization device developed by a group of scientists at the Department of Biomedical Physics & Technology (BMPT), University of Dhaka, that can be heated up water to much more than 60^0C needed to destroy enteropathogenic microorganisms. This solar pasteurizer designed at BMPT automatically allows UV to act on the treated water. So, UV of the sun contributes in destroying diarrhoeagenic microorganisms. Water samples from seven different ponds, lakes and rivers of Bangladesh were subjected to treatment using this device and the highest temperature achieved was 84°C after 4 hours of exposure to sunshine. Regardless of sampling sites, highest reduction of aerobic bacterial population was 4.1±0.7 log CFU/ml recorded in non-selective medium.

In another study, a simple and inexpensive water purification method was sought using natural coagulant (moringa seed powder) and antibacterial agents (scallop powder) followed by natural bio-sand filtration. Surface water collected from different sources (e.g. pond, lake and river) were treated with combined moringa seed powder (0.04%) and scallop powder (0.01%) (MOSP) for 30 minutes showed a clear water layer at the top and a sediment layer at the bottom. The clear water was then passed through natural bio-sand filter and the resulting water was found drinkable. Regardless of sampling sites, highest bacterial population reduction of 5.8±0.9 log CFU/ml was recorded in non-selective medium.

The microbiological and physico-chemical parameters of the water treated with above two technologies showed non-significant differences compared to the United States Environmental Protection Agency (USEPA) drinking water quality parameters. Spiked study and molecular techniques using Polymerase Chain Reaction (PCR) of *uidA* gene of *E. coli* also confirmed the effectiveness of these developed technologies. The shelf-life study of the treated water demonstrated that it can be stored at room temperature up to 6 months without compromising the quality, indicating the usefulness of these two technologies in drinking water scarcity areas

of the world, because the ingredients used are readily available, inexpensive, user friendly and natural. On the other hand, the sediment or sludge produced in the second technique could be used in agriculture field after proper application of compost.

In this study, third technique was using of metals like brass and its constituents, copper and zinc. The results were obtained depending on the mode of the experiment. With brass filings (granules) in a small amount of water such that the water remains entirely within the brass filings, the bacterial count was found undetectable after about 30 minutes of treatment. On having three plates of these three metals at the bottom of three individual plastic containers containing the water under test and shaken continuously for 30 minutes each, if the water volume was small, having a few mm depth above the plates, brass could reduce the bacterial count to non-detectable values. The counts reduced to some extent by copper followed by zinc but were still detectable. When water volume was large with about 50 mm depth above the metal plates, the count reduced for copper to a large extent, but not much for zinc and brass. However, when the treated samples were stored at room temperature, all resident bacteria along with coliform including *E. coli* increased to almost original values after 4 hours. This result indicates that the resident microorganisms were possibly injured due to the effect of copper which resuscitated on storage. Thus, the use of copper, brass or zinc plates were not able to destroy bacterial population with practical volumes of water and therefore, this study was not extended further.

The present work has shown that the solar pasteurization and the technique of using moringa and scallop powder followed by bio-sand filtration are indeed successful in providing safe drinking water in rural areas of the world and despite many similar water purification systems available commercially, these two new techniques would be the simplest, inexpensive and environmental friendly. These simple and easy treatment and filtration methods are particularly helpful for flood prone areas of Bangladesh, where there is a scarcity of drinking water during flood. The people can easily get safe drinking water if training on these techniques are provided to the village people so that they themselves can make the devices when needed, thus empowering themselves.

Key words: Surface water, solar pasteurization device, moringa seed powder, scallop powder, bio-sand filtration, metals, microbial population.

1.0 Introduction

1.1 Introduction

Safe drinking water is the fundamental need for healthy human life and water has been shown linked to the development of all areas including health, nature, urbanization, industrialization, energy production, food security, and equality etc (Colwell *et al.,* 2003). In all these areas, not only water but also clean, pure, safe and quality of water are required to ensure proper development. It is because not only drinking but also exposure to metal or other pollutants in water may cause serious health hazard and even death. Thus, safe water is needed for drinking, domestic uses (like washing, shower, cleaning, cooking) agriculture, and even in industry. At present more than 8,40,000 people per year are dying worldwide because of diarrhea and due to lack of safe water (Huq *et al*., 1996). Although the supply of water seems abundant, more than 97% of the earth's water is salty sea water, while 2% is stored in glaciers, ice caps, and snowy mountain ranges and less than 1% of the earth's water including surface water of lakes, rivers, streams, ponds (0.022%) and groundwater (0.397%) available to us for our daily water supply needs. Furthermore neither all groundwater nor all surface water is easy to reach or clean enough to drink (Yongabi, 2010). In essence, only 0.007 % of the planet's water is thought to be available to feed her 6.8 billion people (Amagloh *et al.* 2009). Thus, water is indeed a valuable resource that is limited to us.

Bangladesh is a riverine country. About 700 rivers are criss-crossed over the country. Nearly 10% (13,830 km^2) of total area of Bangladesh is covered with water, and larger areas are routinely flooded during the monsoon season (Eze *et al*., 2014). Thus, surface water is easily accessible to most parts of the country. However, surface water was being polluted due to disposal of industrial waste, human waste, domestic solid waste and waste of markets and bazaar etc. As a result drinking water from open ponds/rivers contributed to a high level of water-related morbidity and mortality (Sawai *et al.,* 2013).

Thus, safe drinking water is disappearing fast in Bangladesh. According to the UNESCO-sponsored World Water Development Report, published in March 2003, the water supply situation will be worse in the coming decades. Bangladesh will need to increase its agricultural yield about 2% per year to meet the needs of the population that will increase to 200 million by 2020.

To cope with the situation, Bangladesh must rely on surface water from rivers instead of withdrawal of ground water presently being practiced. In order to protect the ground water for emergency uses as well as to ensure maximum use of surface water it is important to introduce a cost-effective, convenient water purification system that can purify surface water to fresh drinking water (Sos arsenic, 2015).

The present well documented technologies used in water treatment such as boiling water for long time, reverse osmosis, ion exchange, UV sterilization, aluminum sulphate and chlorine are becoming un-sustainable, un-ecological, expensive to run, manage and maintain. For example, Boiling of water causes wastage of another valuable natural resource like methane gas, chlorine, which is known to produce trichloromethane, a cancer precursor while aluminum sulphate has been linked to Alzheimer's disease (Desilva 2000; Taraba *et al.*, 1990).

To minimize these drawbacks, three low-cost, sustainable, biodegradable surface water purification techniques have been experimented which are naturally available, non-toxic antimicrobial agents.

Therefore, despite many similar water purification systems available commercially, these new methods would be the simplest, inexpensive and environment friendly. These simple and easy treatment and filtration methods are particularly helpful for flood prone areas of Bangladesh, where there is a scarcity of drinking water during flood. The people can easily get the drinking water if these technologies and training are provided.

1.2 Literature Review

1.2.1 Water

Water is an important and life-sustaining drinks to humans and is essential to the survival of all known organisms. Water is a colorless, transparent, odorless, tasteless liquid found in the world's streams, lakes, oceans, rain and in the fluids of living organisms.(Illustrated Oxford Dictionary, UK, 2007).Water on earth moves continually through the water cycle of evaporation and transpiration (evapotranspiration),condensation, precipitation, and runoff, usually reaching to the sea. Evaporation and transpiration contribute to the precipitation over land (Keys *et al*., 2016). Water used in the production of a good or service is known as virtual water. The Ancient Greek philosopher Empedocles held that water is one of the four classical elements along with fire, earth and air, and was regarded as the ylem, or basic substance of the universe. Plato believed the shape of water is an "icosahedrons" which accounts for why it is able to flow easily compared to the cube-shaped earth (Water science, 2008). Water is considered a purifier in most religions and also considered as a role model in some parts of traditional and popular asian philosophy.

1.2.2 Chemical and physical properties of water

Water (chemical formula H_2O) is a chemical compound in which covalent bond is used to connect one oxygen and two hydrogen atoms. Water appears in nature in all three common states of matter (solid, liquid, and gas) and may take many different forms on Earth: water vapor and clouds in the sky, seawater in the oceans, icebergs in the polar oceans, glaciers in the mountains, fresh and salt water lakes, rivers, and aquifers in the ground.

The major chemical and physical properties of water are:

- Water is a liquid at standard temperature and pressure of 273.15 K (0 °C, 32 °F) and an absolute pressure of 100,000 Pa (1 bar, 14.5 psi, 0.98692 atm). It is tasteless and odorless. The intrinsic color of water and ice is a very slight blue hue, although both appear colorless in small quantities. Water vapour is essentially invisible as a gas (Newscientist magazine, 2010).

- Water is transparent in the visible electromagnetic spectrum. Thus aquatic plants can live in water because sunlight can reach them. Infrared light is strongly absorbed by the hydrogen-oxygen or -OH bonds (Braun *et al*., 1993).
- Water is a good polar solvent and is often referred to as the universal solvent. Substances that dissolve in water, e.g., salts, sugars, acids, alkalis, and some gases – especially oxygen and carbon dioxide (carbonation) – are known as hydrophilic (water-loving) substances, while those that are immiscible with water (e.g., fats and oils), are known as hydrophobic (water-fearing) substances (Campbell *et al*., 2006).
- Pure water has a low electrical conductivity, but this increases with the dissolution of a small amount of ionic material such as sodium chloride.
- The boiling point of water (and all other liquids) is dependent on the ambient pressure. For example, on the top of Mount Everest water boils at 68 °C (154 °F), compared to 100 °C (212 °F) at sea level at a similar latitude (since latitude modifies atmospheric pressure slightly). Conversely, water deep in the ocean near geothermal vents can reach temperatures of hundreds of degrees and remain liquid (Porritt., 2006).
- The density of liquid water is 1000 kg/m^3 (62.43 lb/ft^3) at 4 °C. Ice has a density of 917 kg/m^3 (57.25 lb/ft^3). The maximum density of water occurs at 3.98 °C (39.16 °F) (Kotz *et al*., 2005)
- Water is miscible with many liquids, such as ethanol, in all proportions, forming a single homogeneous liquid. On the other hand, water and most oils are immiscible; usually forming layers with the least dense liquid as the top layer, and the most dense layer at the bottom. Water forms an azeotrope with many other solvents (Milne *et al*., 2006).

- Liquid water can be split by the addition of energy equal to the heat of formation of water in the amount of 285.8 kJ/mol (15.9 MJ/kg). Electrolysis of water is a commonly used method of splitting water into hydrogen and oxygen. The energy required splitting water into hydrogen and oxygen by electrolysis or any other means is greater than the energy that can be collected when the hydrogen and oxygen recombine (Philip *et al*., 2007).

1.2.3 Available sources of water

Water covers 71% of earth's surface, most water is saline (CIA fact book, 2008).Of the total water, 96.5% is found in seas and oceans, 1.7% in ground water, 1.7% in glaciers and ice caps, and 0.001% in the air as vapor, cloud and precipitation. Only 2.5% of this water is fresh water, and 98.8% of the freshwater is in ice (excepting ice in clouds) and groundwater. Freshwater

comprises only less than 0.3% of the total water available to humans. Of that, only 0.06 percent is easily accessible—mostly in rivers, lakes, wells, and natural springs. Even then, accessible water is not necessarily safe drinking water. The freshwater sources from which most of our drinking water is derived are exposed to a variety of contaminants, many arising from the unsafe production, utilization, and disposal of inorganic and organic compounds. Freshwater is available in almost all populated areas of the earth, although it may be expensive and the supply may not always be sustainable. Sources where water may be obtained include groundwater, which clarified by soil and rock layers. Upland lakes and reservoirs, rivers, canals and low land reservoirs are other major sources of freshwater, which have significant bacterial load and may also contain algae, suspended solids and a variety of dissolved constituents. Now-a-days different techniques have been developed for getting freshwater i.e. atmospheric water generation, rainwater harvesting, desalination of seawater and water supply network through tap water.

The most efficient way to transport and deliver potable water is through pipes. Plumbing can require significant capital investment. Some systems suffer high operating and maintenance costs. Because of these high initial investments, many developing nations cannot afford to develop or sustain appropriate infrastructure, and as a consequence people in these areas may suffer hardship for water (Figure 1.1). Over 40 countries in the world suffer from a safe drinking water deficit, with an estimated 1.2 billion people are drinking unclean water on a daily basis and five million people, mostly children are dying every year from water-borne diseases. The United Nations estimates that, by 2025, 2.7 billion people will not have access to safe drinking water. However, three major factors including:

1) untreated municipal and domestic sewage;
2) untreated industrial effluents; and
3) agricultural run-off are attributed to the freshwater crisis in developing countries.

1.2.4 Important human uses of water

Most important use of water for human being is for drinking. Most of the specialists agree to have 2 litres (6 to 7 glasses) of water daily is the minimum requirement to maintain proper hydration (BBC, 2007). Second important use of water is for irrigation in agriculture sector, a key component to produce enough food. Irrigation takes up to 90% of water withdrawn in some

developing countries (WBCSD, 2010). Other important uses of water are in washing, transportation, in chemical uses, food processing, industrial and in recreational purpose.

1.2.5 Worldwide drinking water scenario

About 7% of the world's population has gained access to drinking water during the 13 years since the UN set their Millennium Development Goals, at least a billion still lack access. On top of that, about 2.5 billion people lack adequate sanitation; 35 million people die each year from water-borne diseases. About 2 billion are malnourished and three-quarters of the world's waste water flows to the environment without treatment resulting scarcity of pure drinking water may lead to water borne diseases. Despite economic and social development efforts, the gap between the rich and the poor is growing. By 2050 there will be an estimated 2 billion more people on the planet. Meeting the needs of those people, and the many who already lack access to clean water, is a huge challenge on an already resource-limited planet (Yıldız *et al*., 2016).

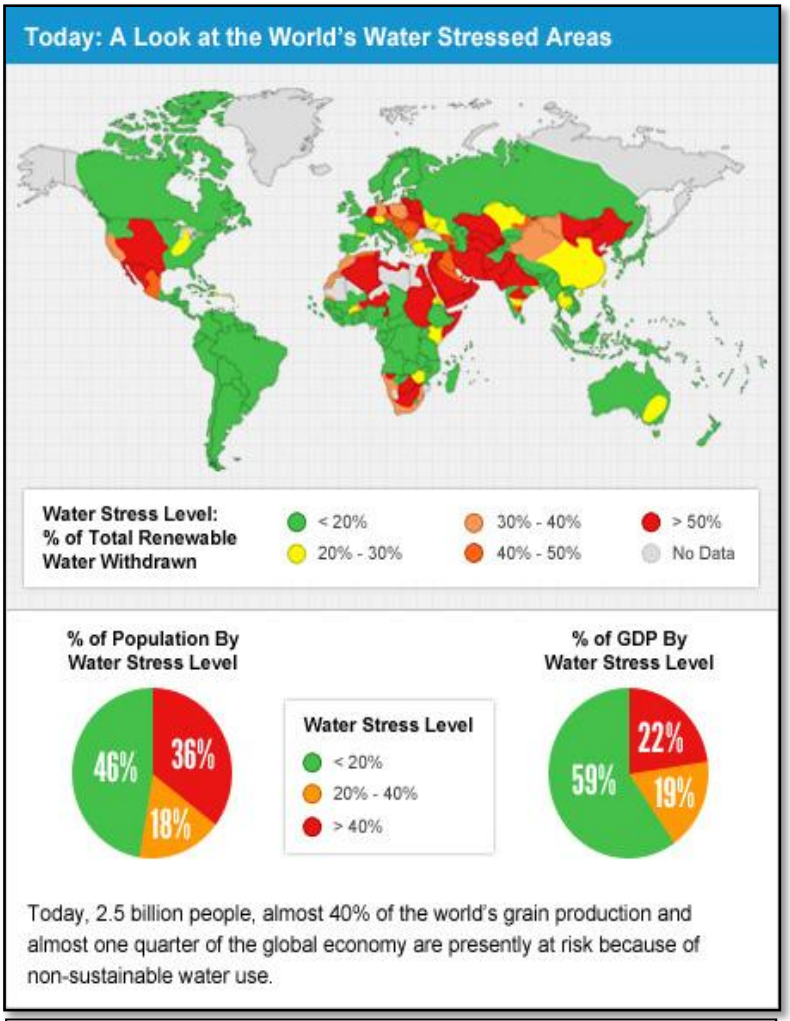


Figure 1.1: World wide water stressed area

Today, many regions of the world are already water stressed due to population and economic growth. In fact, 2.5 billion people (36% of the world population) live in these regions and more than 20% of the global GDP is already produced in risky, water-scarce areas affecting production, the International Food Policy Research Institute (IFPRI), which found that 4.8 billion people – more than half the world's population – and approximately half of global grain

production will be at risk due to water stress by 2050 if status quo, business-as-usual behavior is followed. (Yıldız *et al*., 2016).

By reducing the waste generation and pollution and managing the waste water effectively in individual, collective, agricultural and industrial level, can be achieved higher water productivity levels (economic output per drop) and reduce water stress. On the other hand, continued evolution of technology and infrastructure improvements will enhance water supply capacity for cities and industries while helping deliver clean drinking water and sanitation services to rural populations and the urban poor. Therefore, by changing today's approach to future water management, development of new technology and water productivity can ensure a prosperous future.

1.2.6 Drinking water scenario of Bangladesh

Bangladesh is a riverine country placed in South Asia and formed as a low lying flood delta, with rivers coming from India in the west, Nepal and Himalaya from the north and Myanmar in the east (Van Schendel, 2009).The main sources of water in Bangladesh are surface water, groundwater and rainwater. The Ganges-Brahmaputra-Meghna river system discharges huge amount of surface water through Bangladesh, a part enters into ground to form groundwater. About 93% of the stream flow passing through the country originates from outside the Bangladesh (Ali, 2010). Rainfall within the country contributes to the total water available in Bangladesh, a part of which infiltrates into ground to recharge existing groundwater and the remaining rainwater flows as surface run-off. Surface water is abundant in the wet season in Bangladesh. However, Surface water receives pollutants from agricultural, industrial, domestic and municipal sources. Concentration of silt content in turbulent water in the monsoon is high. Similarly algal growth in stagnant water bodies in the dry season is also very high. Insanitary practices of people have greatly contributed to the deterioration of quality of surface water sources. The biological quality of ponds water is extremely poor due to unhygienic sanitary practices and absence of any sanitary protection. Many of these ponds are chemically and bio-chemically contaminated for fish culture. The deterioration of water quality is directly related to population density and industrial activities due to poor management of domestic and industrial wastewater. The use of surface water for drinking purpose requires clarification and disinfection by elaborate treatment processes. The availability of surface water in the dry season is also a

constraint for the development of dependable small and large scale surface water treatment plants for water supply.

On the other hand, groundwater is the most important source of water supply in Bangladesh. Except for few hilly regions, Bangladesh is entirely underlain by water-bearing aquifers at depths varying from zero to 20 meters below ground surface. Physically groundwater is generally clear, colorless with little or no suspended solids and has a relatively constant temperature. Groundwater is also free from disease-producing micro-organisms which are normally present in large numbers in surface waters. Groundwater in Bangladesh, except in some places, is available at a shallow depth. Groundwater levels are at or near ground level during the period August-October and lowest in April-May. Groundwater rises as a result of recharge during May and usually reaches its highest in late July in each year. Between July and October groundwater levels are constant and maintain a balance between surface water levels and the fully recharged aquifers. The availability of groundwater for drinking purposes has become a problem for the following reasons:

- arsenic in groundwater;
- excessive dissolved iron;
- salinity in the shallow aquifers in the coastal areas;
- lowering of groundwater level;
- rock/stony layers in hilly areas.

Among these problems arsenic in groundwater has become great concern for water supply in Bangladesh. In recent years, the availability of safe drinking water, particularly in Bangladesh's is expected to worsen as the country experiences the effects of climate change. According to a study by the World Bank's water and sanitation programme (2014), about 28 million Bangladeshis, or just over 20% of the population, are living in harsh conditions in receiving clean water (WHO, 2012). The study found that char – land that emerges from riverbeds as a result of the deposit of sediments – is among the most inaccessible, along with hilly areas, coastal regions and haors–bowl-shaped wetland areas in north-east Bangladesh. The struggle to find potable water may intensify during the summer as the surface water sources have already dried up in many parts of the country, which will have a heavy impact on access to drinking water, sanitation and ecosystems. In the drought-prone barind tract area in north Bangladesh, people have to dig more than 350 metres to get safe drinking water. In addition, the situation is

becoming worse due to unusually low rainfall in the areas and underground aquifers are not being replenished.

The water supply in Dhaka city was first started with the establishment of Dhaka Water Works (DWW) by the Nawab Sir Abdul Gani in 1874. Major water works in the sub-continent and even in the developed world started around that time. Although the first water supply in Bangladesh was surface water based, groundwater received priority in the subsequent development of water supply in the country. The surface water treatment plants operated by Dhaka Water Supply and Sewerage Authority (DWASA) in Dhaka and Narayangonj produce about 40 million litres per day (DWASA, 2000). Based on the availability of fresh groundwater, the Department of Public Health Engineering has divided the coastal regions into three types of areas. These are shallow tubewell areas, deep tubewell areas and mixed shallow and deep tubewell areas (Figure 1.2). Shallow Shrouded Tubewells (SSTs) and Very Shallow Shrouded Tubewells (VSSTs) and Pond Sand Filters (PSFs) are alternative options of water supply in the coastal area. Recently, a few rainwater harvesting systems has been constructed by different organizations in arsenic affected areas.

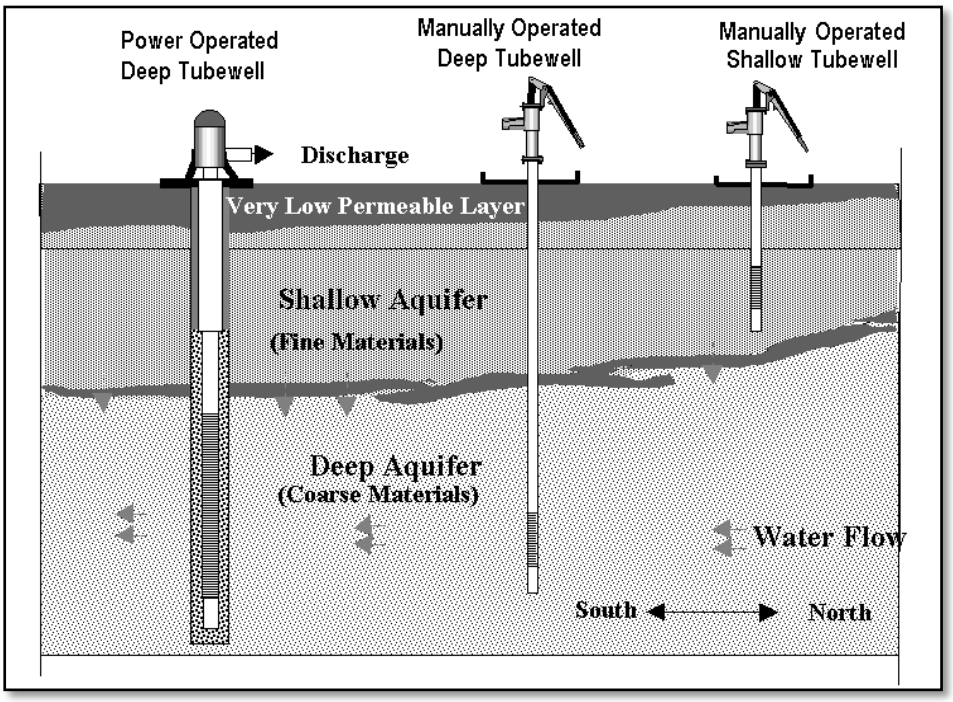


Figure 1.2: The main aquifers and the tubewells for water supply (Harvard University Press, 2000)

Dhaka's underground aquifers are usually recharged with water that percolates underground in nearby districts, but the levels of underground fresh water in those districts have also dropped,

allowing seawater to start seeping into the aquifers. If this continues, experts say, Dhaka's drinking water could become increasingly undrinkable (Thomson Reuters Foundation News, 2013)

1.2.7 Available technologies for drinking water

1.2.7.1 Surface water disinfection technology

Accessibility of clean water, improved sanitation and hygiene can reduce death rate of water borne diseases (WHO, 2013). Therefore, treatment of drinking water is prime importance. Since surface water contains more pathogens than the ground water, so it's crucial to disinfect surface water. Fortunately, there are many techniques to disinfect surface water from microbial contamination, depending on water source and other surrounding parameters.

1.2.7.1.1 Chemical, physical and thermal disinfection methods

Chemical disinfection is generally depend on chlorine based compounds, sodium hypochlorite, solid calcium hypochlorite etc. Some of their concentrations are regulated by USEPA because of carcinogenicity (USEPA, 2016).

Ozone is widely used on cyst form of harmful protozoa (WHO, 2009). Peracetic acid is a mixture of acetic acid and hydrogen peroxide in a watery solution, which can oxidize the outer membrane of microorganisms, causing microbes to be deactivated rapidly.

Most of the surface water requires filtration, as because it contains particles from suspended solids as algae, silt, clay, organic or inorganic materials. Filtration is also effective to remove microorganisms but need to be combined with other treatment processes (WHO, 2008). Granular media filter removes suspended solids or oil, as water passes through the filters. Different types of granular filters are used for surface water disinfection. Among them slow sand filters have slow filtration rate but good filtration technique by using complex biological film (Schmutzdecke) on the surface of fine sand filters (CAWST, 2007). Otherwise, Rapid sand filter removes the particles over a substantial depth within the sand bed. Membrane filtration technique is simply filtering the water through a semi permeable layer but bacteria, chemicals and viruses are prevented from passing. It can remove 0.05 micron particles from water. This technique is effective in the tertiary treatment, when water is used in industry or research works.

Other membrane filtration techniques are nano-filtration, ultra-filtration, micro-filtration and electrolysis (Gollnitz *et al*, 2005).
Coagulation, Flocculation and Sedimentation are another type of disinfection system, in which coagulation and flocculation are applied prior to physical separation of the solid from the liquid phase. The separation is usually achieved by sedimentation or filtration.

Ultraviolet germicidal irradiation technology uses short-wavelength ultraviolet (UV-C) light to kill or inactivate microorganisms by destroying nucleic acids and disrupting DNA and destroy bacteria, viruses, protozoa.

Most popular thermal technologies are boiling or heating water through pasteurization to a certain temperature. In the rural areas of developing countries, like Bangladesh treat the water by boiling using fuel e.g. firewood, charcoal, kerosene (University of Dhaka, 2011).

Solar distillation works on the basis of desalination technique. It uses the solar energy to turn the salt or brackish water into fresh drinking water. Main mechanism is that the influent solution enters the system and more volatile solvent abandon the effluent leaving behind the salty solution. This technique is more energy-intensive; where fresh water is scarce, solar distillation technology has been practiced for many years, principally in tropical and sub-tropical region of earth (Ettouney *et al.*, 2007).

All the earlier mentioned technologies could be used in combination to treat water. The combinations are coagulation and disinfection, media filtration and disinfection and media filtration and membrane filtration.

1.2.7.2 Constrains of the conventional technologies

The above mentioned technologies have failed to meet up the rising demand of safe drinking water because of capital intensive, require huge amount of energy, chemicals and sophisticated equipment, demand of skilled operators. Resources and/or distribution infrastructures lacking make the applications of these technologies extremely limited in developing countries where waterborne diseases are prevalent. Even if these methods are available and affordable, their application could be hazardous to environment or unhygienic when performed by a layperson.

This research work highlighted some of the science and technology to transform contaminated surface water to potable water which is simple, low-cost, sustainable technology without intensive use of energy and chemical compounds.

1.2.8 Effectiveness of innovative low-cost solar pasteurization device in reducing microbial population of surface water

Pasteurization is a process that kills bacteria in liquid food. Solar pasteurization is a process in which water is heated to 60 °C to 75 °C to remove/ destroy diarrhoeal pathogens. The device is the innovation of a group of researchers of Biomedical Physics and Technology Department, University of Dhaka. This device involves the use of polythene sheets or polyethene bags filled with water and other materials available in the rural areas of Bangladesh. These materials consist of a simple device that creates 'Greenhouse-effect' condition. The basic structure is flat plate solar water heater so that the user will get safe drinking water. UV-light also strengthens the technique by destroying the diarrhoeal pathogens at the temperatures which is lower than that required in normal pasteurization technique. Overall goal of this project was to develop a low cost device which is suitable for rural areas and made by readily available materials, which can be used in domestic scale and maintained by the users (Rabbani, 1985 &2012). With this solar pasteurization device, one can disinfect the water as because heat and UV-light contribute to the disinfection of the water. Most dependable factor of this device is weather and the time of the year. During a sunny day, when sunlight intensity is high, the water will heated faster. On a cloudy day, water sample might not reach to the same temperature as sunny day, still UV-light will destroy the pathogens even at lower temperature. (Rabbani, 2002). UV-light destroying the nucleic acid and disrupt the DNA. Then the pathogens are destroyed. Abbott, 2009 reported that, *E. coli* can be destroyed by heating at 65 °C for 45 minutes. The temperature for destroying different microorganisms is presented in the table1.1.

Table 1.1: Duration of exposure at minimum temperature for destruction of diarrhoeal pathogens:

Microorganisms	Disease	Destruction (temp, time)
Salmonella sp.	Typhoid, Paratyphoid	60 °C, 1hour (Feachem *et al.*, 1983)
Vibrio cholerae	Cholera	55 °C, 15 minutes (Hampil,1932)
Escherichia coli	Diarrhoea	65 °C, 45 minutes (Abbott *et al.*,2009)
Shigella sp.	Dysentry	50 °C, 1 hour (Jones *et al.*,2003)
Rota Virus	Child Diarrhoea	50 °C, 30 minutes (Gerba, 1997)

1.2.8.1 The thought of setting up the water disinfection device

The beginning of solar pasteurization device was started with the idea of Professor K Siddique-e Rabbani to do something for rural areas people of Bangladesh. It was 1982 and he worked as a professor of department of Physics, University of Dhaka. That time he started to invent and test different low-cost methods to treat contaminated water in rural areas of Bangladesh. Solar energy is one of the techniques (Rabbani, 2012).

During the design of the water disinfection gadget, the considerable points are:

- The device must cost as little as possible
- The technology should be simple in use
- Materials should be available in rural areas
- It can be used during emergency i.e. during natural disasters
- It should be able to raise the temperature of at least a few liters of water to more than 60 °C (Rabbani, 1985).

The methods are based on pasteurization technique of solar energy. The consisting materials are hay, bamboo, brick, polyethylene etc. which could be found in the rural areas of Bangladesh. Before treating with solar pasteurization device, the surface water first needs to be filtered to remove all floating dirt, algae, mud etc. The filter method consisting of 8 fold of sari (dress worn

by women of Bangladesh).Colwell *et al*, 2003, reported that, the sari could reduce the dirt along with 48% *Vibrio cholerae* of surface water. The setting up procedure of water disinfection gadget is described in the method and materials section.

1.2.8.2 Mode of action of water disinfection device

The green house effect is the main mechanism of this device which increases the water temperature. In the cold countries, people make glass houses, which keep inside warm, while the outside may be freezing. They grow the green plants in that green house. But in our device, we used cheaper and unbreakable transparent polythene sheets instead of glass.

The solar insulation or solar energy that we receive is basically in the form of electromagnetic waves, with wide range of wavelength, ultraviolet-visible-short-infrared-long infrared (Nersesian, 2010).

Polythene and water are transparent to the visible and the short infrared of solar radiation that goes through the polythene and water down to the blackened bottom of the tray. The black layer absorbs al the energy and gets heated up, which in turn heats the water up. Then the heated water in turn gives out energy through long infrared radiation to which polythene sheets are not transparent i.e. these sheets appear opaque to this radiation. Therefore the radiated energy from water cannot come out and is trapped. Through continuous collection of trapped energy, the water temperature keeps going up till it is balanced by heat lost by the system. This process is alike Green House Effect and is shown in the figure 3.

1.2.8.3 The mechanism of heat generation

The heated black layer of the tray heats up the bottom layer of water that is in its contact. Hot water is lighter than cold water, therefore this hot water rises and cold water from the top sinks, which gets heated as it comes in contact with the black surface below. The hot water at the top cools by losing heat to air above, and becoming heavier, comes down, again to get heated up at the black bottom. Thus a loop current builds up and the whole of the water heats up. This process is called convection and also indicated in the figure 1.3. The hot water cannot lose heat by radiation as mentioned above, but loses heat through other processes called conduction and convection to the outside environment. The temperature to which the water can be raised depends on the balance of the heat gained and heat lost. With stronger sunshine, more heat will be gained, and higher will be the temperature. On the other hand, loss of heat will decrease the

temperature. Therefore, to achieve high temperature, heat loss will be minimized through this two other processes. A thick hay bed is placed underneath to prevent heat loss through conduction to the ground below. The hay bed has trapped air pockets which makes it a good insulator of heat. On the top side, there had been nothing above the hot water, it would have lost heat quickly through convection to the air above (air heated by hot water would rise up and cool air would come down forming a loop, similar to that described above for convection in water). Since one also need sunlight to enter from the top, difficult situation being created. Minimization of the heat loss make-up by creating two thin layers of air using 3rd and 4th polythene sheets (using some straw strands to prevent the sheet from touching). The second polythene sheet touching the water surface is required to prevent water from evaporating and condensing in the form of water droplets under the polythene sheet above, which would have blocked sunlight. In this arrangement, sun's ultraviolet radiation also enters and acts as an additional agent to destroy diarrhoeal pathogens.

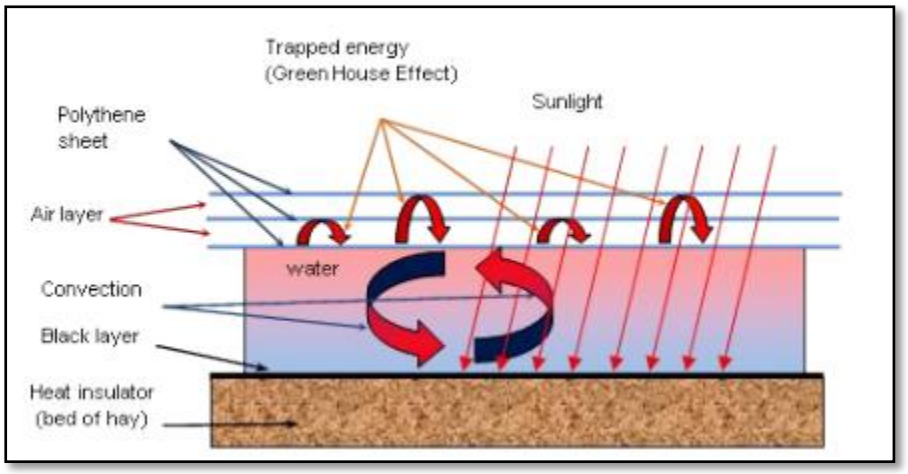


Figure 1.3: Schematic picture of solar water pasteurization device mechanism

1.2.8.4 The way to know the achieved desired temperature

In the laboratory scale experiment we used a mercury thermometer, to know the achieved temperature. But in the rural areas, there is no facility of a thermometer. Then we found a low cost indicator, which acts like a maximum recording thermometer. Some crushed wax was added in a 3 cm square transparent polythene sheet and the open end was sealed. Then it has been placed between 2nd and 3rd sheet of polythene sheet. Wax melt at about 55 °C, and if the water reaches this temperature, the wax in the bag will melt and make it almost transparent. This can be understood even if the water cools down later. By twisting the bag molten and solidified wax can again be crushed to a powder and reused.

1.2.9 Effectiveness of moringa seed powder, scallop powder and biosand (sand-charcoal-gravel) filter in the purification of surface water

In order to ensure safe water for human consumption, numerous water purification methods were developed. Some are really expensive; some are not that much reliable, and some have further adverse effects on health. For decades, commonly used methods for treating water are reverse osmosis, ion exchange, UV irradiation, coagulation with aluminum sulphate (Alum) and iron (II), chloride etc. The purpose of all these treatments is to remove organic and inorganic particles from the water before consumption (Yongabi *et al.,* 2010). Recent studies claim that these methods are becoming untenable, uneconomical, eco-hazardous and most importantly, unhealthy. Excessive use of coagulant chemicals has dreadful consequences on health like cancer, neurological disorders etc (Amagloh *et al.,* 2009; Aho *et al.,* 2012; Mangale *et al.,* 2012).

Figure 1.4: *Moringa oleifera* tree

1.2.9.1 Moringa seed powder

To circumvent the use of chemical agents to purify water, scientists have started focusing on natural products which can be used to treat water (Amagloh *et al.,* 2009). Varieties of plants and their different parts were screened for coagulation and antimicrobial activity (Mangale *et al.,* 2012). Such one useful plant is *Moringa oleifera* (drumstick) which is commonly known as drumstick pods (figure 1.4). Numbers of studies have been carried out to establish the

coagulation activity of the seed of this plant (Choubey *et al.*, 2012; Ali *et al.*, 2009; Kalikawe *et al.*, 2015).

The *Moringa oleifera* plant is 5-12 meter in height. It looks like an open, umbrella-shaped crown with long trunk and corky, white colored bark. Usually this plant grows well in low humidity (Ali *et al*., 2009) and has tuberous taproot. The plant produces deciduous leaves and white or cream colored fragrant flowers. The pods are enclosed with light wooden shell and consist of around 20 seeds and 3 papery wings (Aho *et al.*, 2012; Eze *et al.*, 2014). The seed kernel of this plant has considerable amount of low molecular weight, water soluble proteins. These proteins are characterized as positively charged in solution. After adding to water, the positively charged proteins primarily magnetize negatively charged particles (clay, silt, bacteria, and other toxic particles). As a result, large flocs are formed by continuous agitation. Thus, the flocs are removed by settling or filtration (Aho *et al.,* 2012). The seed also serves as antimicrobial agent as it contains few antibiotics like, benzyl isothiocyanate and benzyl glucosinolate. These antimicrobial peptides either disrupt cell membrane or inhibit essential enzymes (Mangale *et al.*, 2012).According to Ali *et al,* (2009), *Moringa oleifera* seed shows high coagulation activity in highly turbid water and vice versa. On the contrary, Kalikawe *et al*, (2015) reported that this seed powder is more efficient in low turbid water. The efficacy of seeds depends on source and quality of raw water. After treating water with *Moringa oleifera* seed, it is noted that total hardness, turbidity, acidity, alkalinity, chloride are decreased at significant level (Mangale *et al.*, 2012). Till now, there is no indication of potential health risk and toxicity (Amagloh *et al.,* 2009; Mangale *et al.*, 2012; Eze *et al.*, 2014) from *Moringa oleifera* seed.

1.2.9.2 Scallop shell powder

Another environmental element that has been come to focus is scallop shell powder. It is considered that this material possesses high antibacterial activity. Scallops are harvested in a large quantities in Korea, Japan etc. Some parts scallop are utilized as food additives, plastering and paving materials etc but a huge quantity of shells are dumped. They are known as commercial waste products in these countries. In most cases, after harvesting, the scallop shells are piled on the sea-shore (Bae *et al*., 2006). The major constituent of scallop shell is $CaCO_3$,which gets converted to CaO through heat treatment at around 700ºC and then exerts antibacterial activity (Sawai *et al*., 1999, 2001, 2003).Mechanism of antibacterial effect of scallop powder is dependent on the making of alkaline environment and production of reactive oxygen species which cause the bacterial inhibition (Hou *et al*., 2016).

Previous studies demonstrated that heated scallop shell powder treatment successfully decreased bacterial count in shredded cabbage, lettuce, frankfurters, and destroy *Bacillus subtilis* spores &*E. coli* (Sawai *et al*., 2001; 2003; Kim *et al*., 2006; Kubo *et al*., 2013).

It improves shelf life of potato salad and controls growth of coliform on wild warabi plant too (Choi *et al*., 2006; Bodur *et al*., 2010). According to Sawai *et al*.,(2001), $CaCO_3$ and CaO do not have any mutagenic effect. Rather CaO helps in lessening mutagenicity. Moreover, if heated shells are dumped in open air, CaO changes to $CaCO_3$ by reacting with CO_2. So the use of scallop shells would be a great solution in food industry to increase shelf life of foods as well as a significant means of way out to environmental pollution (Bae *et al.*, 2006; Sawai *et al.*, 2013; Watanabe *et al.*, 2014).

1.2.9.3 Bio-sand (sand-charcoal-gravel) filter

Another constituent part of this technique is bio-sand filter, which is a point-of-use for water treatment system adapted from traditional slow sand filters. Bio-sand filters for this study were typically constructed using PVC pipe with a tightly fitted lid to prevent contamination and unwanted pests from entering the filter. The clear water from upper portion was then passed through a 3-step natural bio-sand filtration specially designed in PVC pipe (Figure1.5). Main constituents of this filter are sand, charcoal and gravel layer. Water sample passes through sand, charcoal and gravel and comes into contact with organic matter, and the presence of charcoal may absorb some color and stone might add some minerals to the water (stauber *et al*., 2006).All the constituents are locally available, cheap and environmental friendly, so that rural areas people can easily avail this filter. Buzunis, 1995 also has been studied about the sand-charcoal-gravel filter and reported that the filter can remove faecal coliform about 96% in laboratory studies.

When the water sample poured into the filter, at first it travels through the sand column, which removes bacteria including *E. coli* and other bacteria upto 96.5% (Elliott *et al*.,2015), Virus removal of about 70 to 99% including echovirus 12 and different bacteriophages (Elliott *et al*, 2008) and different types of suspended solids. Early studies also reported (Palmateer *et al.*, 1999) that it has the ability to remove *Cryptospordium* sp.and *Giardia lamblia* oocysts. A study of stauber *et al*., 2006 reported that, bio-sand filtration can lessen the occurrence of diarrhoeal diseases and improve the quality of water. Then the water sample travels through charcoal layer, which is called, activated charcoal, because after treating with oxygen, the carbon opens up millions of tiny pores between the carbon atoms. Activated charcoal works mainly as adsorbent,

which has been recommended as one of the best available technologies (Mukherjee *et al.,* 2007) and by catalytic reduction, the process involves the attraction of negatively-charged contaminant ions to the positively-charged activated carbon. Organic compounds are removed by adsorption and residual disinfectants such as chlorine and chloramines are removed by catalytic reduction. Activated carbon removes/reduces odor, many volatile organic chemicals, pesticides, herbicides, as well as chlorine, benzene, trihalomethane (THM) compounds, different solvents, man-made chemicals which are found in tap water (Taraba *et al.,* 1990).A gravel layer is present below the charcoal column. The gravel layer gives the water additional minerals.

1.2.10 Low-cost technology for inactivation of diarrhoeal pathogens of surface water using metals

Technology for safe drinking water for rural areas of the low resource countries is still a big challenge for the humankind. The present study was designed to develop a simple technology, employing the bactericidal effect of metals that can be implemented by rural households. The research was initiated by the report of Koneczny *et al*., 2012, that brass door knobs in hospitals contained less germs than on single metal knobs, which was supplemented by the knowledge of the ancient practice in rural Bangladesh of leaving water in a brass or bronze pitcher overnight.
Copper and silver ions together have been known to have a bactericidal effect since ancient times, although the mechanisms are only surfacing in the recent times. Copper silver ionization was developed in both Europe and the United States in the 1950's. There has been a renewed interest in the use of copper and silver nanoparticles embedded in various base materials and have been claimed to disinfect water. (Jain *et al*., 2005 ; Lin *et al*., 2013;Morones *et al*., 2005;Oyanedel *et al*, 2008; University of Virginia,2014).

The department of Biomedical Physics and Technology have developed simple low-cost solar pasteurization techniques for providing diarrhoeal pathogen free drinking water, with the target of providing techniques suitable for households in rural areas and which can be made by the rural people themselves with materials available easily in the local market or in their surroundings (Rabbani,1985; 1992; 2002). Therefore, instead of going for metallic nanoparticles, which may be difficult to procure in a rural environment, the department went for using direct metal plates or their granules and the present group was formed for this investigation.

Different types of metals have been used for inactivation of diarrhoeal pathogens, i.e. copper, zinc and their alloy, brass. At first, the research work initiated with brass filing, because brass is the alloy of copper and zinc metal. The copper in brass makes brass germicidal. Depending on type and concentration of pathogens present, brass kills microorganisms within a few minutes to hours of contact. Antimicrobial effect of copper and copper alloy such as *E. coli*, *Mycobacterium tuberculosis*, methicillin resistant *Staphylococcus aureus*, influenza A virus, *Salmonella typhi* and *Vibrio cholera* have been reported in various study(Godbole, 1971; Klarriech, 2001; Noyce *et al.,* 2006; 2007; Mehtar *et al.,* 2008; Sudhaa *et al.,* 2009; Dhanalakshmi *et al.,* 2013).

1.2.11 Microbiological analysis

Microbiological analysis of water is a method of analyzing treated or untreated water to estimate the number of microbes present and, if needed, to find out what sort of microbes they are. Among them bacteria, viruses and protozoa are mostly important.

- **Bacteria:**

Aeromonas spp., *Campylobacter* spp., *Clostridium* spp., *Escherichia coli* (including VTEC types such as 0157), *Legionella*, *Leptospira* spp., *Pseudomonas aeruginosa*, *Salmonella enterica*, *Shigella* spp., *Vibrio* spp. and *Yersinia* spp.

- **Viruses:** Adenoviruses, Hepatitis A, Noroviruses, Poliovirus, Rotaviruses.
- **Protozoa*:*** *Cryptosporidium* spp., *Giardia* spp.

Testing for all these pathogens directly is not practical, partly because of the difficulty and expense of conducting the comprehensive testing on large numbers of water samples, but also because, in heavily contaminated water, pathogens tend to be present in a very low number. Since, most of the above listed species are not present in water as a result of human and animal wastes, so it is usual to examine water samples for evidence of such faecal contamination by testing for the presence of so called "indicator" bacteria. Indicator organisms are commonly used to assess the microbiological quality of surface waters and faecal coliforms (FC) are the most commonly used bacterial indicator of faecal pollution (Antony *et al.,* 2012).The presence of these indicator organisms in water is evidence of faecal contamination and, therefore, of a risk that pathogens are present. If indicator organisms are present in large numbers, the contamination is considered to be recent and/ or severe (UNEP/WHO, 1996).

1.2.11.1 Commonly used indicator microorganisms

Earlier three indicator organisms were recommended for assessing the quality of drinking water. These are:

1) Total coliform (TC) bacteria.

2) Faecal coliform (FC) bacteria and

3) *Escherichia coli*

Other bacteria that can be considered as secondary indicators, they are proposed, but not yet internationally recommended are as follows:

4) Faecal *streptococci*

5) *Pseudomonas* spp.

6) *Clostridium perfringens*

7) *Bifidobacterium* spp.

8) *Bacteroides fragilis* group

9) *Rhodococcus coprophilus*

10) Bacteriophages/Coliphages and Virus

Recently, *Aeromonas* spp, and *Legionella* spp. have attracted much attention to be considered as indicators of bottled water quality. Kabir *et al.,* 2012 reviewed the usefulness of yeast *(Candida albicans)* as an indicator. Bacteriophages have also been evaluated as indicators, particularly for enteric viruses (Scarpino *et al.,* 1978). According to some investigators *Bacteroides* spp. can be regarded as an indicator (Espinosa *et al*., 2009; Fong, 2005; Harwood *et al.*,2005).

1.2.11.2 *Escherichia coli*

Escherichia coli is a member of the family Enterobacteriaceae, and is characterized by possession of the enzymes β-galactosidase and β-glucuronidase. It grows at 44–45 °C on complex media, ferments lactose and mannitol with the production of acid and gas, and produces indole from tryptophan. However, some strains can grow at 37 °C but not at 44–45 °C, and some do not produce gas. *E. coli* does not produce oxidase or hydrolyse urea (Fotadar *et al*., 2005). Complete identification of the organism is too complicated for routine use, but a number

of tests have been developed for rapid and reliable identification. Phenotypic and genotypic tests have been standardized at international and national levels for *E. coli* identification and accepted for routine use; others are still being developed or evaluated. Outbreak of diseases involving consumption of drinking water contaminated with *E. coli* from human and animal faeces, sewage have been reported (Cabral, 2010; Farnleitner *et al.,* 2010).Recently, it has been suggested that *E. coli* may be present or even multiply in human faeces of tropical water(Ishii *et al.,* 2006; Goto *et al.,* 2011). However, even in the remotest regions, faecal contamination by wild animals, including birds, can never be excluded (Harwood *et al.,* 2005).

Animals can transmit pathogens that are infective in humans, the presence of *E. coli* or thermotolerant coliform bacteria must not be ignored, because the presumption remains that the water has been faecally contaminated and that treatment has been ineffective (Cabral., 2010)

1.2.12 Water quality testing methods

Water quality tests are typically dependent on microbial presence, especially faecal coliform bacteria, and physico-chemical parameters. Several internal factors affect these parameters. These parameters have the major influences on biochemical reactions that occur within the water. Sudden changes can cause the indicative change of water conditions (McLellan, 2004; Anderson *et al*., 2005). Heterotrophic and indicator bacteria (coliform) belonging to the enterobacteriaceae are commonly used as indicator for microbial water quality. Assessment of faecal population in waters is generally achieved by determining the number of faecal coliforms that present in the sample. The faecal coliform counts are reliable indicator for assessing the sanitary quality of water because of some coliform genera, such as *Citrobacter* spp, *Klebsiella* spp and *Enterobacter* spp. also some coliform and non faecal coliform genera (Anderson *et al.,* 2005).

On the basis of indicating public health risk of infection or disease from exposure to microbial contaminated water, the direct measurement of a pathogen or a reliable microbial indicator of pathogens by culture or infectivity is generally considered the "gold standard," and both should be the goal of any new measurement technique (Mandal *et al.,* 2011; Stevens, 2003).

1.2.12.1 Cultural techniques

- **Liquid enrichment method**

Various methods have been developed for analyzing the pathogens by culture or infectivity, among them, the most commonly used culture methods for bacteria are colony counts on membranes or agar medium plates or liquid broth culture (London *et al.,* 2010).

- **Membrane-filtration method**

Membrane filtration is a technique that uses a physical barrier, a porous membrane or filter, to separate particles in a fluid. Particles are separated on the basis of their size and shape with the use of pressure and specially designed membranes with different pore sizes (Han *et al.*, 2008).Nanofiltration, ultrafiltration, microfiltration, and reverse osmosis are all membrane filtration techniques.

- **MPN method**

The most probable number (MPN) is the number of organisms that are most likely to have produced laboratory results in a particular test. The MPN method is used to quantify the concentration of the viable microorganisms in a sample and involves inoculating decimal dilutions into tubes of a broth medium, observing results and using a standard MPN table (Sutton, 2010).

- **Presence–absence tests**

Presence–absence tests may be appropriate for monitoring good-quality drinking-water where positive results are known to be rare. Thus, presence–absence tests are not recommended for use in the analysis of surface waters, untreated small-community supplies, or larger water supplies that may experience occasional operational and maintenance difficulties (Clark *et al.,* 1993).

- **Plate count method**

There are two ways of performing a plate count: the spread plate method and the pour plate method.

a) Spread Plate method

The number of bacteria in solution can be readily quantified by using the spread plate technique. In this technique, the sample is appropriately diluted and a small aliquot is transferred to an agar plate. The bacteria are then distributed evenly over the surface by a special streaking technique. After colonies are grown, they are counted and the number of bacteria in the original sample calculated (Hartman, 2011).

b) Pour plate method

In a pour plate, a small amount of inoculum from a broth culture is added by pipette to the centre of a Petri dish. Cooled, but still molten, agar medium in a test tube or bottle is then poured into the Petri dish (Sanders, 2012).

1.2.12.2 Molecular techniques

A number of methods have been developed that exploit the sequence divergence among taxa to examine microbial community structure. The culture independent methods for microbial community analysis most often utilize Polymerase Chain Reactions (PCR) to amplify phylogenetic markers in DNA extracted from microbial community. Most molecular approaches utilize the 16s rRNA gene as a phylogenetic marker because of its genetic stability and composition of conserved and variable regions (Case *et al.*, 2007).

The establishment of public databases of 16s rRNA gene sequences (http://rdp.cme.msu.edu/ and http://www.ncbi.nlm.nih.gov/) from cultured organisms allows for taxonomic classification of uncultured bacteria. To examine the microbial community, a number of fingerprinting approaches have been developed. These approaches for bacterial identification are based on sequences from variable regions of the 16s rRNA gene. The most frequently used techniques include Terminal-Restriction Fragment Length Polymorphism (T-RFLP), Denaturing Gradient Gel Electrophoresis (DGGE) and cloning and sequencing. These methods take the advantage of the conserved regions of the 16s rRNA gene as they utilize PCR primers binding sites that are present in all bacteria (Jansson *et al.*, 2011).

1.2.12.2.1 Nucleic Acid based techniques

- **Polymerase Chain Reaction**

Polymerase Chain Reaction (PCR) is an in situ DNA replication process that allows for the exponential amplification of target DNA in the presence of synthetic oligonucleotide primers and a thermostable DNA polymerase (Garibyan *et al.,* 2013). A wide range of different concentrations or units of DNA templates, Taq DNA polymerase, primers, and temperature cycles have been employed for detection and quantification of microorganisms found in soil and water. Other components of a PCR reaction such as deoxyribonucleotide triphosphates (dNTPs), magnesium (Mg^{2+}) and buffer solutions have been used in different concentrations to increase detection limits.

A PCR process may involve the use of one primer (single PCR) or multiple primers (multiplex PCR) to detect bacterial isolates. Other forms of PCR are real-time PCR, nested PCR, reverse-transcription PCR and many more.

Polymerase chain reaction assays have been routinely used for rapid detection, identification and differentiation of foodborne pathogens. They have been used in are such as DNA cloning, diagnosis of hereditary and infectious diseases, identification of genetic fingerprints, and detection and diagnosis of infectious diseases. Polymerase Chain Reaction technique plays an important role in the identification of typical bacterial strains that exist in viable but non-culturable coccoid forms (e.g. Campylobacter spp.) which are often missed by the conventional method (Adzitey *et al.,* 2013).

The technique can be expensive and its sensitivity and performance can be inhibited by components of enrichment broth and DNA extraction solution, concentration of the PCR mixtures (primers, DNA templates, dNTP's andMg^{2+}), and temperature and cycling conditions (Dietrich *et al*., 2013; Kalle *et al*., 2014).

Other available methods:

- Denaturing Gradient Gel Electrophoresis (DGGE)/Temperature Gradient Gel Electrophoresis (TGGE),
- Terminal-Restriction Fragment Length Polymorphism(T-RFLP),
- Fluorescent in situ hybridization (FISH)
- Sequencing of 16S rRNA genes

1.2.13 Physico-chemical parameters

It is very essential and important to test the water before it is used for drinking, domestic, agricultural or industrial purposes. Water must be tested with different physico-chemical parameters. Some physical test should be performed for testing of its physical appearance such as temperature, color, odor, pH, turbidity, TDS etc, while chemical tests should be performed for its BOD, COD, dissolved oxygen and other characters. For obtaining more and more quality and purity water, it should be tested for its trace metal, heavy metal contents. It is obvious that drinking water should pass these entire tests and it should contain required amount of mineral level. Only in the developed countries all these criteria's are strictly monitored. Due to very low concentration of heavy metal and organic pesticide impurities present in water it need highly sophisticated analytical instruments and well trained manpower. Following different physico-chemical parameters are tested regularly for monitoring quality of water.

- **Color**

Color is not a toxic characteristic, but is listed by the EPA as a secondary (aesthetic) parameter affecting the appearance and palatability of water (Wedgworth *et al*., 2014)

- **Taste and Odor**

The primary sources of taste and odor problems in drinking water are algae and bacteria. The United States Environmental Protection Agency (USEPA) has issued a secondary maximum concentration limit (MCL) limiting the TON to 3. However, the primary regulator with respect to taste and odor is the consumer (Francis *et al*., 2015).

- **Temperature**

Temperature is a measure of the intensity (not the amount) of heat stored in a volume of water measured in calories and is the product of the weight of the substance (in grams), temperature (^{o}C) and the specific heat (Cal g - ^{o}C-1) (Hosseinlou *et al.*, 2013).

- **pH**

pH is most important parameter in determining the corrosive nature of water. Lower the pH value higher is the corrosive nature of water. USEPA standard of pH is 6.5 to 8.5 (Kulthanan *et al.*, 2013).

❖ **Salinity**

Salinity is a measure of the content of salts in soil or water. Salts are highly soluble in surface and groundwater and can be transported with water movement. There are two main methods of determining the salt content of water: Total Dissolved Salts (or Solids) and Electrical Conductivity method.

❖ **Total Dissolved Solids (TDS)**

The TDS consist of the material left in a vessel after evaporation of filtered sample and include different kinds of nutrients and minerals which are considered as useful parameters in determining the productivity of reservoir. USEPA standard is 500mg/L (Kumar *et al.*,2012).

❖ **Turbidity**

Turbidity is a measure of the degree to which the water loses its transparency due to the presence of suspended particulates. Turbidity is measured in NTU: Nephelometric Turbidity Units. The instrument used for measuring it is called nephelometer or turbidity meter, where the USEPA standard is 0.5-1 NTU (Schwartz *et al*., 2000).

❖ **Biochemical Oxygen Demand (BOD)**

BOD is a measure of organic material contamination in water, specified in mg/L. BOD is the amount of dissolved oxygen required for the biochemical decomposition of organic compounds and the oxidation of certain inorganic materials (e.g., iron, sulfites). Typically the test for BOD is conducted over a five-day period. USEPA standard is 5mg/L (Gregg *et al*., 2013)

❖ **Chemical Oxygen Demand (COD)**

COD is another measure of organic material contamination in water specified in mg/L. COD is the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water. USEPA standard is 40mg/L (Hur *et al*., 2010).

❖ **Arsenic**

Arsenic in drinking-water is a hazard to human health. It has attracted much attention since recognition in the 1990s of its wide occurrence in well-water in Bangladesh. It occurs less extensively in many other countries also. The main source of arsenic in drinking-water is arsenic-rich rocks through which the water has filtered. It may also occur because of mining or industrial activity in some areas. USEPA standard for drinking water is 0.01mg/L (Guo *et al.*, 2007).

❖ **Iron and Manganese**

Iron and manganese are non-hazardous elements that can be a nuisance in a water supply. Iron is the more frequent of the two contaminants in water supplies; manganese is typically found in iron-bearing water. USEPA standard of drinking water is 0.3 and 0.05 mg/L for iron and manganese respectively (Gantzer *et al*., 2009).

❖ **Lead**

Lead is a toxic metal that is harmful to human health; there is no safe level for lead exposure. Drinking water is only one of the possible routes of exposure to lead contamination, but it is one of the easiest routes of contamination to reduce. The primary route for lead poisoning is the distribution system used to carry water. USEPA standard of drinking water is 0.015 mg/L (Brown *et al*., 2012).

❖ **Zinc**

Zinc in drinking water is found through groundwater contamination. USEPA standard about zinc in drinking water is 5mg/L (Sparks *et al*., 2006).

❖ **Cadmium**

Drinking-water from shallow wells of areas in Sweden where the soil had been acidified contained concentrations of cadmium approaching 5 µg/l. USEPA standard of drinking water is 0.005mg/L (Arain *et al*., 2015).

1.2.14 Spiked/Inoculation/Challenge test

It is an intentional application of known number of microorganisms in test samples. The results evidenced that the actual number of reduction of microorganisms in any test sample. It is also named as spiked/ inoculation study (Beuchat *et al*., 2001; Inatsu *et al*., 2003).

The fact behind the challenge test is the environmental bacteria are exposed to different stressed conditions, either by physical or chemical treatment. Therefore cells are injured either structurally or metabolically. Structural damage results in loss of permeability of cell wall and cell membrane. Metabolic damage results in damage of functional components such as ribosomes, structural DNA and enzyme inactivation. After injury, cells lose their ability to replicate. If the damage is not severe, then the cells can multiply after some period (Brashears *et al*., 2001).

Recovery of stressed *E. coli* has been widely studied (Ahmed *et al*., 1995; McCleery *et al*., 1995; Rocelle *et al*., 1995; Taormina *et al.*, 1998). Different types repaired methods have been documented, depending on types of food, pathogen of interested, procedure of inoculation and test conditions, retrieval of pathogens and also the way of reporting results (Beuchat *et al*., 2001). Among these, two most common methods are the Liquid-Media methods and Solid-Media methods (Brashears *et al*., 2001). In the Liquid-Media methods, nutritionally complex nonselective liquid medium involves with incubation period of 1 to 5 hours. This technique uses MPN (most probable number) techniques for enumeration. Otherwise, Solid-Media method use direct plating (either surface or pour plating) with nutritionally rich nonselective medium and incubated at 25 to 37 °C for 1 to 4 hours to facilitate repair. Then the plates are overlaid with selective agar medium specific for the type of microorganism and incubated under specific time and temperature.

Another method commonly used to evaluate the effectiveness of recovered injured cells is the use of antibiotic resistant strains of pathogens. In this method, non selective media containing antibiotic is used (Brashears *et al*., 2001). Nalidixic acid, rifampicin and streptomycin have been widely used as effective selective agents to distinguish inoculated strains from other co-existing bacterial flora (Inatsu *et al*., 2003). Only a few validated studies have been reported of the application of drug resistant strains (Beuchat *et al*., 2001).

1.2.15 Detection of *uidA* gene of *E. coli*

United States federal regulations stated that public water systems must conduct analysis for fecal coliforms or *E. coli* for any routine drinking water sample which is positive for total coliform bacteria (U. S. Federal Register, 2010). Consequently, new media has been proposed for the detection of these microorganisms. The most commonly used fluorogenic substrate for the detection of *E. coli* is 4-methyl umbelliferyl-β-D-glucuronide (MUG). The substrate detects the activity of β-glucuronidase (GUD), which is the first enzyme of hexuronide-hexuronate pathway in *E. coli* and is encoded by *uidA* gene (Martins *et al.*, 1993). Detection of the pathogen in water using conventional culture and biochemical assays is highly time consuming (18-96 hours), arduous and not reliable. Another significant constraint of *E. coli* is that the number of them can be altered in sample within less time periods. As a result, detection by exploiting ß-glucuronidase activity of *E. coli* may give false positive or false negative result. Moreover, viable but non-culturable cells also remain uncountable in conventional culture method (Khan *et al.*, 2007). To crack the hindrance in detecting pathogens in water, molecular techniques come

up as blessings. Use of polymerase chain reaction and agarose gel electrophoresis has turned out to be the most effective tools for detection. To detect *E. coli*, several genes are targeted for PCR amplification such as, lacZ and lamb, *uidA*, 16s rRNA, gadAB and cyD. Among these genes, the housekeeping gene *uidA* is mostly used. While PCR is conducted to identify *E. coli*, specific primers for *uidA* gene are used (Heijnen *et al.,* 2006; Khan *et al.*, 2007).However, Mcdaniels *et al*., (1996) suggested that, genotypic assays for the *uidA* gene are more sensitive in detecting *E. coli* isolates from water samples than the Mug based phenotypic Assays for β-glucuronidase (Mcdaniels *et al.,*1996).

1.2.16 Scanning Electron Microscopy

The scanning electron microscope uses a focused beam of high energy electrons over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition. The microscope reveals the information about the sample including external morphology (texture), chemical composition, crystalline structure and orientation of materials making up the sample.

In most applications, data are collected over a selected area of the surface of sample, and a two dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 micron in width can be imaged by SEM (magnification ranging from 20X to approximately 30,000 X, spatial resolution of 50 to 100nm).

Figure 1.5: Scanning Electron Microscopy

1.2.16.1 Principles of Scanning Electron Microscopy (SEM)

When the electrons are accelerated, that can carry kinetic energy, and the energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. The signal include secondary electrons (that produce SEM

images), backscattered electrons, photons, visible lights and heat. Secondary electrons and backscattered electrons are morphology and topography on samples and backscattered electrons are most valuable for illustrating contrast in composition in multiphase samples. X-ray generation is produced by in elastic collisions of the incident electrons with electrons in discrete shells of atoms in the sample. As the excited electrons return to lower energy state, they yield X-rays that are of a fixed wavelength. Thus characteristic X-rays are produced for each element in a mineral that is 'excited' by the electron beam. SEM analysis doesn't lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly (McGregor *et al.*, 2012).

1.2.17 Pyrogen test

During cell division and lysis of cells, endotoxin released. Endotoxin is a part of lipopolysaccharide complex that forms the outer envelope of gram negative bacteria (Henderson *et al.,* 1996). Endotoxin is an essential part of quality assurance and quality control involves the testing end product of clinical and raw materials (Rosimar *et al*., 2004).

As because human body is highly sensitive to minute amount of endotoxins and gram negative bacterial infections can often cause a pyrogenic response. The presence of endotoxin can cause fever, inflammation and irreversible shock. Higher concentration of endotoxin can cause irreversible shock observed in cases characterized by fulminating gram negative bacteria.

1.2.17.1 Conventional pyrogen tests

A) Rabbit pyrogen tests

Originally, endotoxin testing and pyrogen testing were performed since 1942, when it was introduced into the USP (United States Pharmacopoeia). The rabbit pyrogen test was discovered by Seibert, 1925. The presence of pyrogens leads to an increase in the body temperature of rabbits. Contamination of pyrogens is hazardous, especially in drugs for intravenous use. However, some exogenous pyrogens are heat stable, sterilization is not sufficient to remove the fever causing agent. In the 1940s, rabbit pyrogen test was introduced for detection of fever causing contamination, and was consequently incorporated into various pharmacopoeias and guidelines.

B) Bacterial endotoxin tests

Bacterial endotoxin test (BET) in the pharmacopoeias describes different types of tests which primarily detects endotoxins in gram- negative bacteria. Their appearance results in the clotting reaction of the amoebocyte lysate of horseshoe crabs. The test is historically referred to as the Limulus Amoebocyte Lysate (LAL) test, although lysates from both Limulus polyphemus and Tachypleus tridentatus are employed (Joiner *et al*., 2002). The LAL test has been replaced by the rabbit pyrogen test over the last 30years. There are various types of methods for measuring LPS-induced reaction (i.e. clotting reaction, kinetic turbidimetric measurement, chromogenic endpoint and kinetic reaction), which show some dissimilarities (Meisel, 1995). As because, LAL test only detect the endotoxin of gram negative bacteria, so it has generally been possible to assure "pyrogen-free" products with the LAL test. Availability of endotoxin standard that permits the semi-quantitative or quantitative measurement of endotoxins is the main advantage of LAL test. Detection limit of LAL test is 0.03IU/ml. However, potentiality of individual endotoxin differs in LAL and mammalian assays. LAL test is undoubtedly suitable for the pyrogen testing of a number of products, but not for all products. For example, certain biological drugs interfere with the LAL test, and therefore still tested by using rabbit pyrogen test. The LAL test is not applicable to the detection of endotoxins absorbed onto inorganic or organic surfaces. Positive reaction in LAL test indicates the product is pyrogenic for humans. Otherwise, negative result doesn't mean that the product is pyrogen-free, since this test cannot detect non endotoxin related pyrogen in the sample.

There are three different methods for this test.

I. Gel-Clot Technique

Gram negative bacterial endotoxin catalyzes the activation of a proenzyme in the LAL. The initial rate of activation is determined by the concentration of endotoxin present. The activated enzyme (coagulase) hydrolyzes specific bonds within a clotting protein (coagulogen) also present in LAL. Once hydrolyzed, the resultant coagulin self-associates and forms a gelatinous clot. Now, gel clot method is often thought to be most sensitive and accurate procedure of testing endotoxin content in pharmaceutical injectable products.

II. Turbidimetric technique

This technique is a photometric assay measuring increase in reactant turbidity. In this technique, an activated clotting enzyme cleaves a clotting protein and the cleavage product coalesces as a

result of ionic interaction of cleavage and cause the reaction mixture to turbid. The technique is an endpoint-turbidimetric assay or a kinetic-turbidimetric assay.

III. Chromogenic Technique

This technique was developed in 1977 by Japanese investigators who determined that endotoxin-activated LAL would cleave amino acid cleavage sites containing chromogenic peptides. That method is based on the cleavage of the peptide coagulogen, which is caused by a cascade of enzyme activation steps. The insoluble cleavage products include coagulogen and coagulin, which coalesce by ionic interaction. When a sufficient amount of coagulin is present, the test solution becomes turbid and forms a gel clot. When endotoxin is present in a sample, components of LAL are activated in a proteolytic cascade that results in the cleavage of colorless artificial peptide substrate liberating p-nitroaniline, which is yellow and its absorbance is 405 nm. The test is performed by adding a volume of Pyrochrome to a volume of specimen and incubating that reaction mixture at 37 °C (Joiner *et al*., 2002).

C) Human Whole Blood Test

This technique, using Enzyme-Linked Immune Sorbent Assay (ELISA) was developed in 1996 by Hartung and Wendel (Hartung *et al.,* 1996) and validated by European Centre for the validation of Alternative Methods (ECVAM) internationally (Hoffman *et al*., 2005). In the technique, human monocytes face a challenge with pyrogens and produce cytokines in vitro. The cytokines can be measured by specific ELISA tests. This reaction is quantitative, that is, the more pyrogens are in the sample, the more cytokines are produced. It is less expensive and more sensitive than the rabbit pyrogen tests. Advantage is the reaction strength is directly in human materials. Unlike the LAL, the test can detect not only endotoxins, but also lipoteichoic acids, fungi and superantigens such as SEB (enterotoxin of *Staphylococcus aureus*) (Schindler *et al.,* 2007).

1.2.18 Reasons behind carried out this research work

The rural areas of Bangladesh generally depends on low quality of water from rivers, ponds and/or streams for their daily life purposes, thereby making them prone to water borne diseases. So, this research work highlighted some of the science and technologies to transform contaminated surface water to potable water which are simple, low-cost, sustainable technology without intensive use of energy and chemical compounds.

1.3 Aims and Objectives of the Study

Most of the surface water sources are microbiologically of low quality which is in need of treatment prior to drink. Different types of conventional methods as chemical precipitation and filtration, coagulation, disinfection, softening, pH regulation, oxidation and reduction, electrochemical treatment; reverse osmosis and ion exchange are used to improve the water quality. However, these methods are economically non-sustainable due to their capital intensive; requirement of huge amount of energy, chemicals and sophisticated equipments, demand of skilled operators respectively. Low-cost synthetic coagulants of aluminum, ferric salts and soda ash are widely used for water treatment, but the effect of these chemicals on health issue and safety is increasingly becoming questionable. In rural areas, the problem tends to be serious due to high level of illiteracy, communication infrastructure and lack of social amenities. Such communities in these localities depend on low quality of water from rivers, ponds and/or streams for their daily life purposes, thereby making them prone to water borne diseases. So, the search for alternative low-cost, effective, eco-friendly methods of water treatment is therefore timely.

The study focuses on some of the science and technologies to transform contaminated surface water to potable water which are simple, low-cost, sustainable technology without intensive use of energy and chemical compounds.

1.3.1 Specific objectives

The present study encompasses the following specific objectives:

- Evaluation of the microbiological and physico-chemical quality of surface water treated with–
    a) Low-cost solar pasteurization device
    b) Combination of moringa seed powder, scallop powder followed by bio-sand (sand+ charcoal+ gravel) filtration
    c) Use of metals (Brass, Copper, Zinc).
- Phenotypic characterization of the environmental isolates on the basis of cultural and biochemical analysis.
- Evaluation of the treatment efficiency by cultural technique and molecular technique by PCR assay of *uidA* gene of *E. coli.*

- Analysis of actual number of reduction of specific microorganism (marker bacteria) in water samples by inoculation/spiked study.
- Assessment of cytotoxic effect of moringa seed powder and scallop powder using tissue culture methods.
- Detection of pyrogenic substances of control and treated water samples by Limulus Amoebocyte Lysate (LAL) tests.
- Scanning electron microscopy of single and eight folded cotton sari (which was used for straining of water sample prior to treatment).

2.0 Materials and Methods

This research work mainly involves assessment of the microbiological and physico-chemical parameter of water samples treated using several low-cost techniques innovated in recent days micro and macro water purification systems. Molecular analysis of indicator bacteria, *E. coli* for *uidA* gene has also been evaluated using Polymerase Chain Reaction. Details on the media compositions, chemical reagents, primer sequences and apparatus used in this study are presented in appendices section.

2.1 Sampling sites

Seven sites including local ponds, lakes and rivers were selected for collection of surface water in order to process them with the proposed technology. Three ponds located within the Dhaka University campus were selected. Dhanmondi Lake situated at a very populated area of Dhaka city was also chosen as a site of collection. Three rivers Shitalakshya at Narayangonj, Padma at Manikgong and Meghna at Bhola district were also chosen as sampling sites.

2.1.1 Water samples: During sample collection, samples were collected from a little distance of ponds and rivers avoiding immediate vicinities of the edges. The water samples were collected in pre-sterilized glass bottles or PET bottles from relatively fresh flow and from a depth of 4-6 cm. Standard procedures were followed for sampling (APHA, 1992). After collection, water samples were transported to the laboratory in an insulated foam box with ice to maintain a temperature ranging from 4-6 °C.

2.2 Microbiological and physico-chemical analysis

Prior to treatment, microbiological and physico-chemical analysis were conducted for control water samples. For microbiological analysis, water samples were added to cultural media. The physico-chemical parameters include temperature, pH, color, odor, taste, salinity, turbidity, TDS, conductivity, BOD/COD and metal and heavy metals include iron, manganese, lead, zinc, arsenic, and cadmium which were analyzed. Global Position System (GPS) coordinates of the sampling sites were measured by a GPS navigator (eTrex Legend HCx, Garmin, Taiwan) and given in Table2.1.

Table2.1: GPS coordinates of seven sampling sites

Sampling site	GPS coordinates
Shahidullah Hall Pond, University of Dhaka	23°43'32.5" N 90°24'09.5" E
Jagannath Hall Pond, University of Dhaka	23°43'45.0" N 90°23'36.0" E
Bangla Academy Pond, University of Dhaka	23°43'32.5" N 90°24'09.5" E
Dhanmondi Lake, Dhaka city	23°44'34.5" N 90°22'37.0" E
Shitalakshya River, Narayangonj district	23°37'09.8" N 90°30'29.2" E
Padma River, Manikgong district	23°15'47.0" N 90°36'20.5" E
Meghna River, Bhola district	22°47'51.8"N 90°39'51.8"E

2.3 Innovated technologies proposed for treatment of water

2.3.1 Low-cost solar pasteurization device developed by a group at the Department of Biomedical Physics & Technology, University of Dhaka.

2.3.2 Combination of moringa seed powder, scallop powder followed by bio-sand (sand+ charcoal+ gravel) filtration technique developed by a group at the Centre for Advanced Research in Sciences (CARS), University of Dhaka.

2.3.3 Use of metals (Brass, Copper, Zinc), technique developed by a group at the Department of Biomedical Physics & Technology, University of Dhaka.

2.3.1 Low-cost solar pasteurization device

Materials needed for preparing the device:

1. Hay (paddy straw/grass straw): about 1.5 kg.

2. A bamboo tray about 75 cm in diameter, painted black inside using enamel paint (or a similar circular or square tray made of any other material, including plastics or metals).
3. Four transparent and thick polythene sheets each of which is at least 40 cm larger than the diameter of the tray. Transparent polypropylene is still better.
4. Some heavy weights to press onto the polythene sheets outside, to keep them stretched.

- **Setting up of the low-cost solar pasteurization device (Figure 2.1)**

1. The hay was spread out as a bed to a thickness of at least 10 cm and the bamboo tray was placed over it. The hay bed prevents heat from escaping below.
2. Straining water through a piece of clean cloth is an extremely simple, low- resource method and widely used for household water treatment. Cloth filters have been used in many cultures for centuries. Typically in South Asia, a sari (a strip of unstitched cloth ranging from four to nine meters in length that is draped over the body of women, it is also a traditional garment) is folded 3 to 4 times and used as a filter.
3. All polythene /polypropylene sheets were cleaned well.
4. The first polythene sheet was spread on the tray and water was poured to a depth not more than 2 cm. The water will get heated quicker if the depth is less. In a tray of 75 cm diameter, about 5 liters of water will make the specific depth. The same depth of water all throughout has been ensured by adjusting the hay below.
5. The second polythene sheet was spread so that it touches the water surface everywhere. If there is any air bubble, then it was removed to the sides by lightly pushing with a finger. Otherwise, water vapour will condense at the position of the bubble and will block sunshine.
6. Then a few strands of straw were spread on the second ploythene sheet and a third polythene sheet was spread on top. A layer of air is necessary between the 2^{nd} and 3^{rd} sheet to prevent the escape of the heat upwards, and the strands of straw are used to ensure that the sheets don't touch anywhere. Too many straw strands will block sunshine and so was avoided.
7. Similarly a few strands of straw were spread on the 3^{rd} sheet and the fourth and final sheet was spread at the top. Again, the strands of straw are to prevent the 3^{rd} and 4^{th} sheets from touching.

8. Now to keep all the sheets stretched, some weights were put on the polythene sheets outside the tray. One can use bricks or pieces of wood or make a ring of a thick rope out of straw or jute for the purpose. Ina clear midday sunshine, it has taken about 1.5 to 2 hours to heat the water above 60 °C and thus to destroy the diarrhoeal pathogens.
9. The top three polythene sheets were removed and the edges of the bottommost sheet were picked up together for collecting the diarrhoeal pathogen free water. Then the treated water was poured carefully into a storage pitcher or water tank. To avoid contamination, it should be maintained strictly that the user hands should not touch the water or the part of sheet through which water may pour out.
10. The microbiological and physico-chemical analysis of the treated water was done to find out the quality of the treated water, whether it was safe to drink or not (figure 2.1).

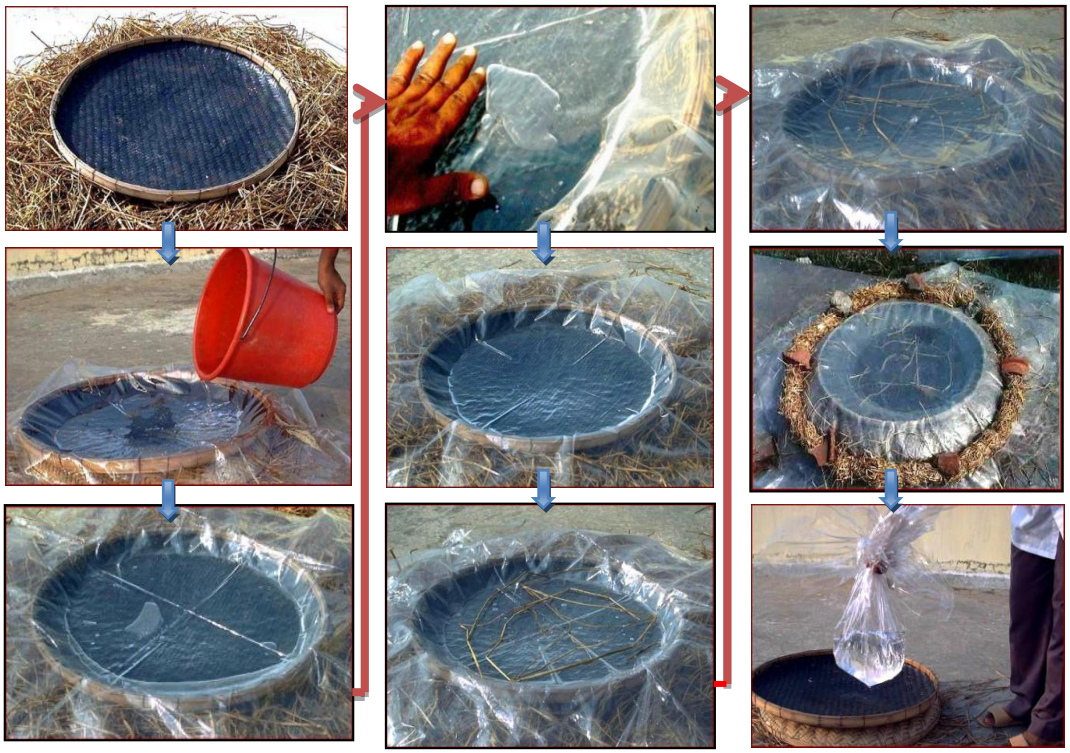


Figure 2.1: Setting up of solar pasteurization device

2.3.2 Methodology of combined moringa seed powder and scallop powder followed by bio-sand filtration

1. One of the cheapest ways of removing turbidity is by filtering the turbid water with cotton clothes. These fabrics are cheap and readily available, which make them perfect materials for water treatment in rural areas. Cotton sari clothes (a traditional garment of unstitched cloth

ranging from four to nine meters in length that is draped over the body of women) is folded over three to four times (8 layer) and water sample was passed through the folded sari. Both non-filtered and filtered water was analyzed for physico-chemical and microbiological analysis.

2. Moringa seed powder of 0.4 gm and 0.1gm of scallop powder were added in 1000 ml of strained water in a tumbler, stirred vigorously with a glass rod for five minutes and the content was left for 30 minutes to settle down the sediments.

3. Bio-sand filter is a water treatment system which was adapted from traditional slow sand filters. Bio-sand filters for this study were constructed using PVC pipe with a tightly fitted lid to prevent contamination and unwanted pests from entering the filter. The treated water from upper portion was then passed through a 3-step natural bio-sand filter specially designed using PVC pipe (Figure 2.2). The top of the filter is a sand column (12 cm), below the sand column is a layer of charcoal (12 cm) followed by a layer of gravel (12 cm). At the end of sand and charcoal layer 2-fold sari (cotton cloth) was placed that prevents sand particles to enter into charcoal column and charcoal particles to gravel layer. When water is poured into the filter, it travels through the sand column, which removes pathogens and suspended solids, and then travels through charcoal layer, which removes odors and chemicals, and below the charcoal column, a layer of gravel (add minerals) prevents charcoal from entering the drainage layer and clogging the outlet tube. Below the separating layer is the drainage layer consisting of coarser gravel that prevents clogging near the base of the outlet tube (5 liter of pure water).

4. The microbiological and physico-chemical analysis of the treated water was done to know whether the water could be drinkable. Laboratory scale and household scale treatment device was presented in the figures 2.3 and 2.4 respectively.

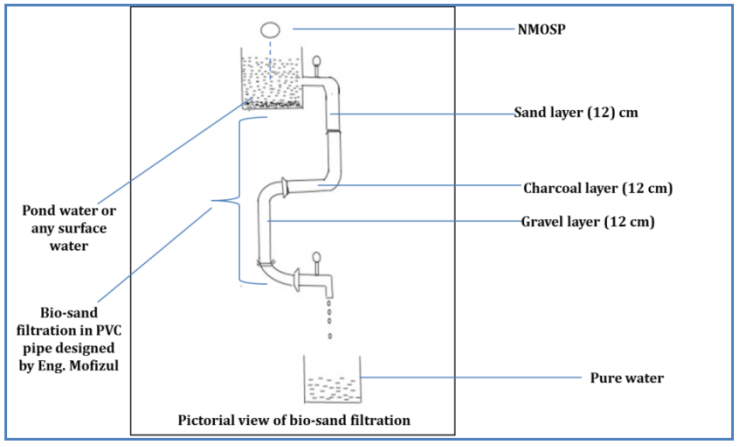


Figure 2.2: Pictorial image of bio-sand filtration

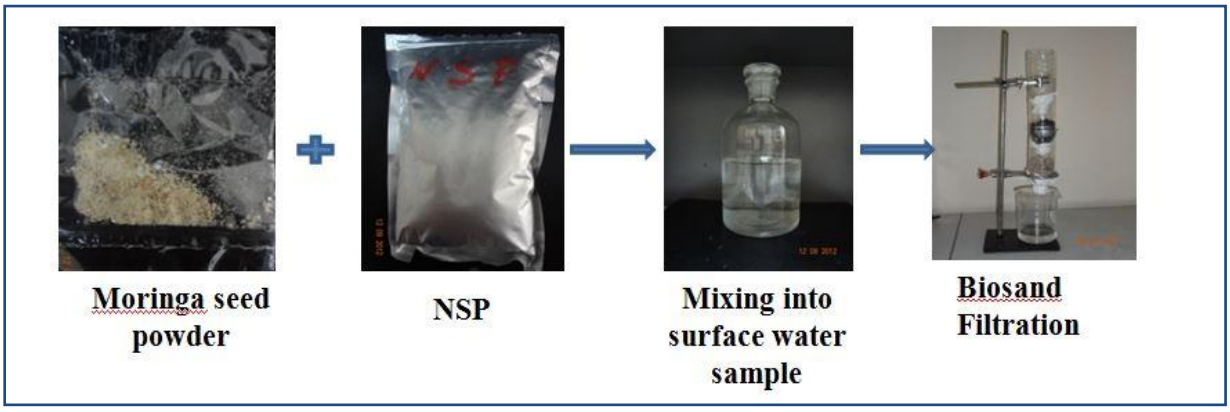


Figure 2.3: Laboratory scale moringa seed powder, scallop powder followed by 3-step bio-sand filtration treatment device

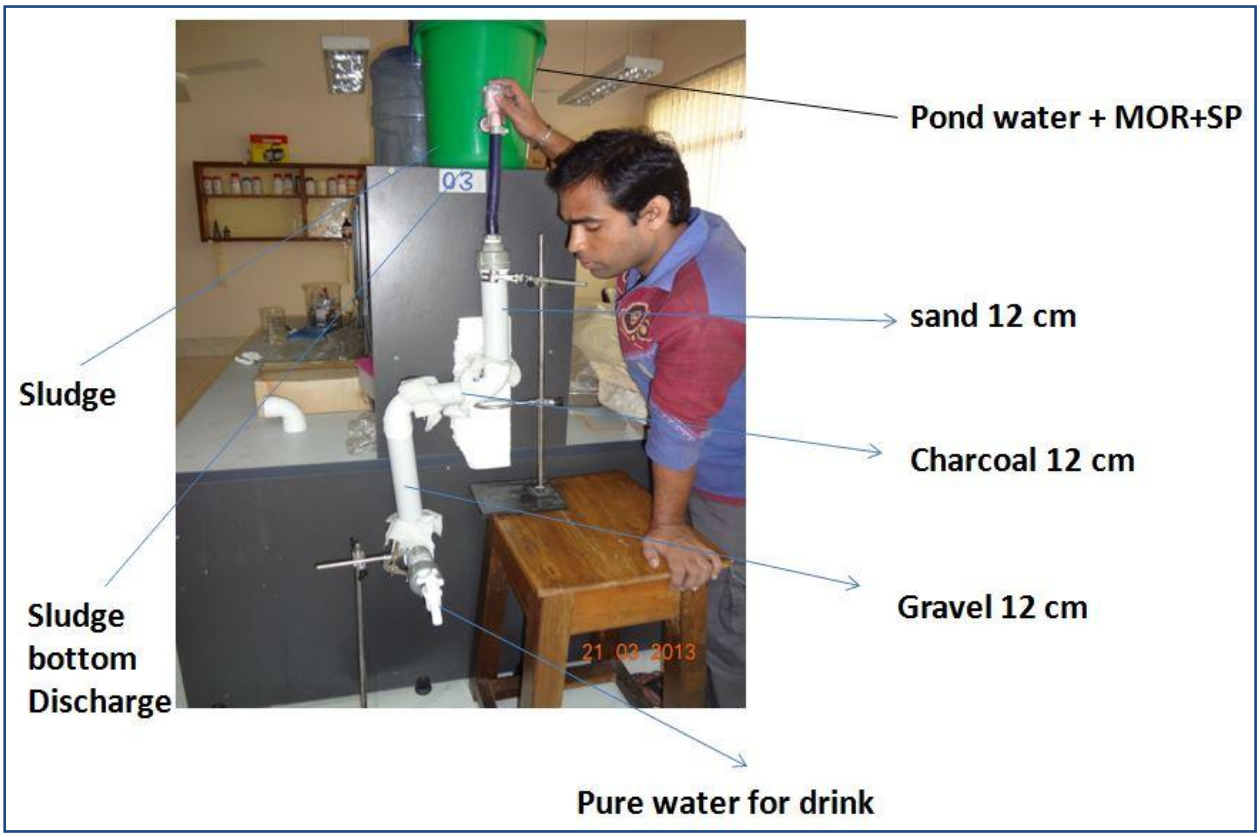


Figure 2.4: Household scale moringa seed powder, scallop powder followed by 3-step bio-sand filtration treatment device.

2.3.3 Simple metals in inactivation of diarrhoeal pathogens of surface water

I. **Use of Brass granules:** one **(**1.0g) of brass filing was poured into a test tube. Water sample of a measured volume (5/50/100 ml) strained using the cloth filter was added to the test tube and then shaken gently. Then the solution was left for 30 minutes. After that

the treated water was separated from brass filing and microbiological analysis of treated water was done.

II. **Use of brass, copper and zinc plates:** After that, the research work has been expanded to two constituents of brass, copper and zinc, and three sheets of these three metals were used for the treatment (Figure 2.5).

1. Brass, copper and zinc metallic sheets of 11cm×7cm sized were placed in three plastic containers of fitting size.
2. 50/100/400 ml of filtered water was added to each container.
3. Then the containers were left for different period i.e. 24, 48 hours.
4. Microbiological analysis of treated water was done in an attempt whether the water could be drinkable.

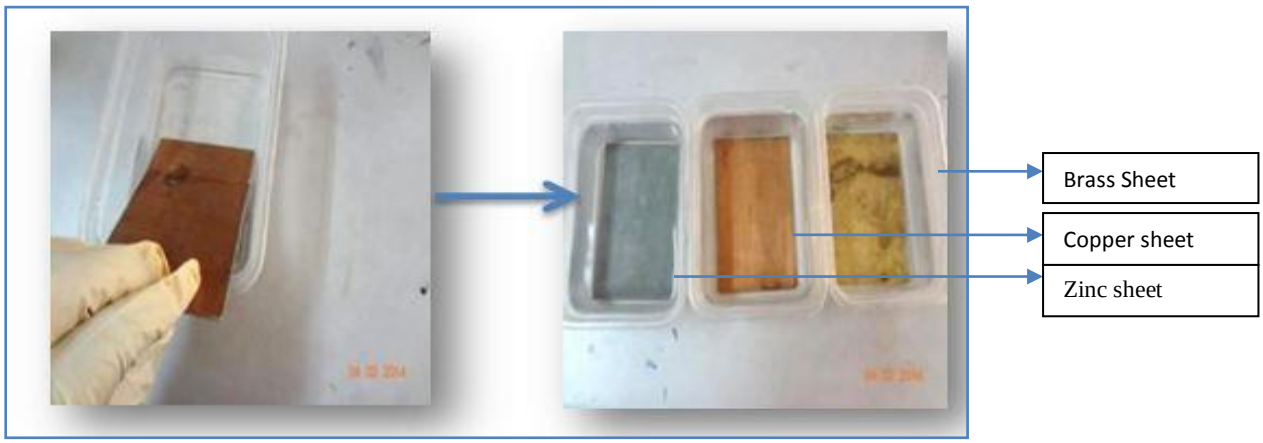


Figure 2.5: Experiment with brass, copper and zinc plates

III. **Effectiveness of copper inactivation in the long term:** Second step experiments revealed that, copper was the best in inactivation of *E. coli*. So, next step was evaluation of the effectiveness of copper plate in inactivation of diarrhoeal pathogens in the long term. The methodologies are:

1. Two copper plates of 11cm×7 cm were placed in two plastic containers of fitting size.
2. 400ml of filtered water was added to each container.
3. One container was left in a cool and dry place at room temperature with copper plate for 24 hours (microbiological study was done after 24 hours), while another one was used for periodic microbiological study of 1, 2, 4 and 24 hours of treatment with copper plates.

2.4 Methodology of spiked study/inoculation study/challenge study (Figure 2.6)

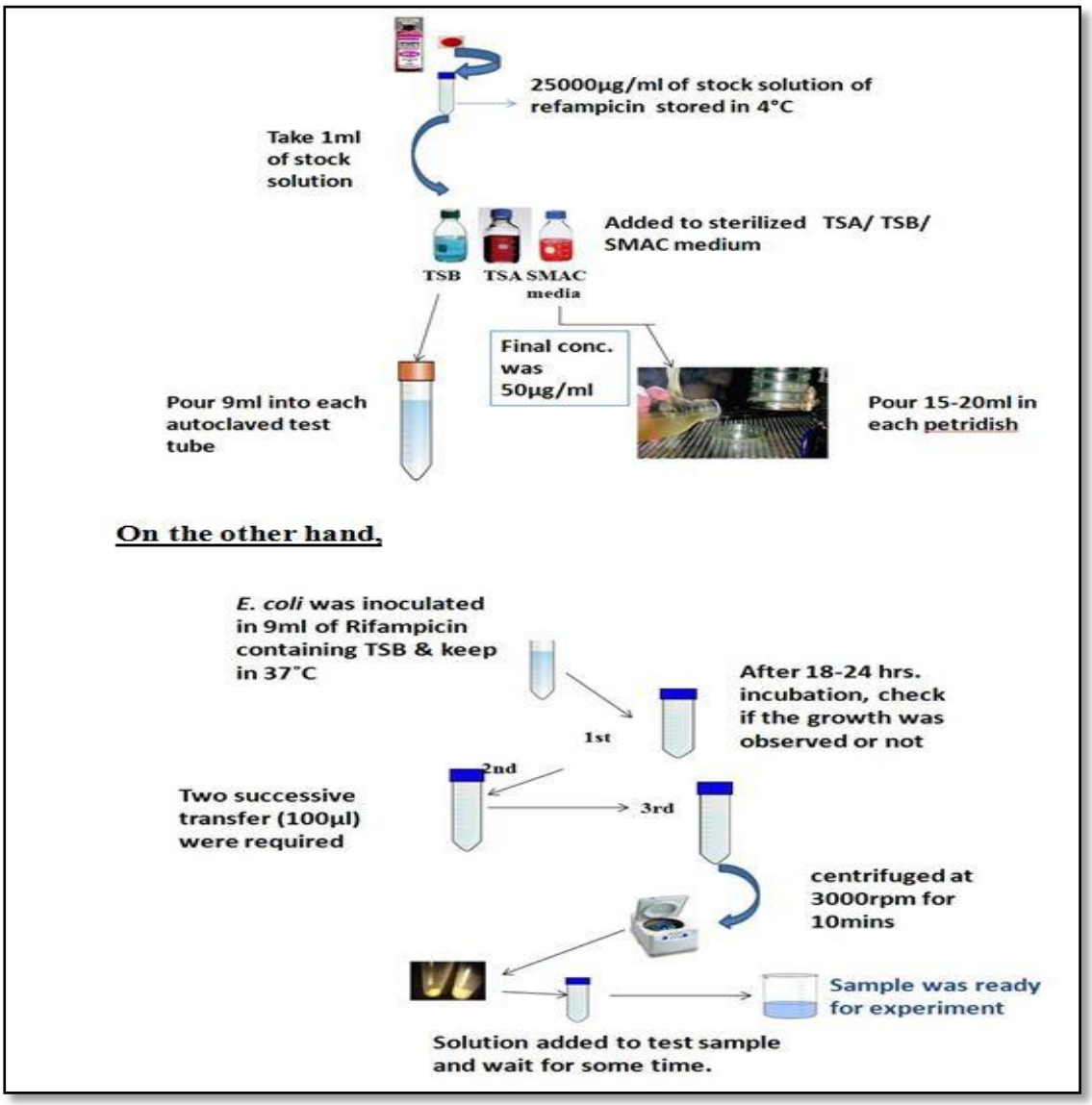
1. *E. coli* strain isolated from surface water was used in this study. The test strains were adapted to grow in Tryptic Soy Broth (Sigma Chemical Co. Ltd, St Lewis, UK) (PH 7.3) supplemented with rifampicin (TSB-Rif ; 50µg/ml).
2. Cultures were transferred to TSB-Rif by loop at three successive 24-hr intervals before they were used as inocula.
3. Cells were then collected by centrifugation (3,000 rpm, 5 minutes) washed and re-suspended in sterile normal saline.
4. The lower inoculums of 10^3 CFU/ml and higher inoculums of 10^8 CFU/ml concentrations was prepared and applied to the filtered pond water within 1 hour of preparation and research experiments were conducted.
5. 

Figure 2.6: Methodology of spiked study (Beuchat *et al*., 2001; Inatsu *et al*., 2003)

2.5 Microbiological analysis

2.5.1 Cultural and biochemical approaches

2.5.1.1 Presumptive identification using cultural technique: The bacteriological analysis includes total aerobic bacteria, total coliform, fecal coliform and presence of *E. coli*, *Salmonella* spp. and *Vibrio* spp. were done according to the USFDA Bacteriological analytical methods (2001). Spread plate technique on selective and non-selective agar media was used to identify specific bacteria. Tryptic Soy Agar (TSA, Fluka, USA) for total aerobic microbial count, Hichrome^{TM} Coliform Agar (Fluka, USA) for total coliform count, Sorbitol MacConkey Agar (Oxoid, UK) for *E. coli*, Bismuth Sulphite Agar (BD, USA) for *Salmonella* spp., and Thiosulfate-Citrate-Bile salts-Sucrose Agar (TCBS) for *Vibrio* spp., were used in this study. All the agar plates were incubated at 35-37 °C for 24-48 hours before being counted. At least 5 randomly picked presumptive colonies of *E. coli*, *Salmonella* spp. and *Vibrio* spp. were isolated from selective agar and confirmed using biochemical test and API 20E kit (bioMerieux sa, Marcy-l'Etoile, France) (http://apiweb.biomerieux.com).

2.5.1.2 API 20E

The API 20E system facilitates the 24-hour identification of Enterobacteriaceae as well as 24 or 48-hour identification of other Gram negative bacteria. The API 20E strip consists of micro-tubes containing dehydrated substrates for the demonstration of enzymatic activity and carbohydrate (CHO) fermentation. The substrates were reconstituted by adding a bacterial suspension. After incubation, the metabolic end products were detected by indicator systems or the addition of reagents. CHO fermentation is detected by colour change in the pH indicator (Figure 2.7). Here, JS-20120829 strain of *E. coli* was used as positive control. Basic procedures are:

i. A bacterial suspension was prepared in 5ml of sterile normal saline (0.85% NaCl) of 0.5 Mc Farland Standard.
ii. An incubation tray and lid is supplied for each strip. 5 ml of water was dispensed into the tray and the specimen number was recorded on the elongated flap of the tray.
iii. After placing the strip in the incubation box, the tube section of the microtubes were filled by placing the micropipette tip against the side of the cupule.
iv. ADH, LDC, ODC, H_2S, AND URE microtubes were slightly underfilled.
v. Both of the tubes and cupules section of [CIT], [VP] and [GEL] tubes were filled.

vi. After inoculation, the cupules section of the ADH, LDC, ODC, H2S and URE tubes were completely filled with sterile mineral oil.

vii. The plastic lid was placed on the tray and the strip was incubated for 18-24 hours at 35 ^{o}C in a non-CO_2 incubator.

viii. After incubation the following reagents were added to their respective compartments:

- 1 drop of Kovac's reagent to IND
- 1 drop of Barritt's A and B (VP1 and VP2) reagent to VP
- 1 drop of $FeCl_3$ (Ferric chloride) to TDA

ix. The results were recorded on the result sheet and checked out from internet.

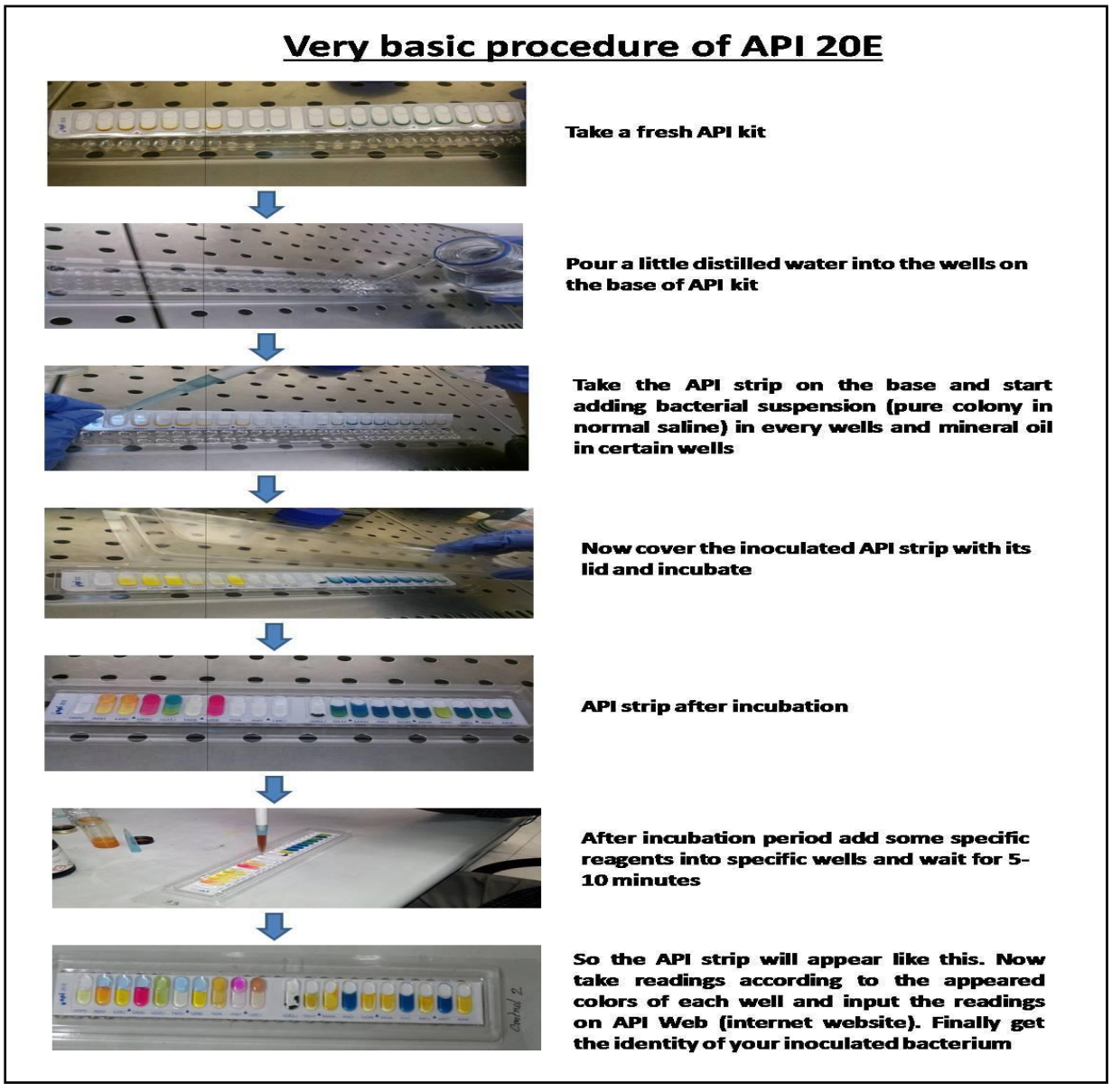


Figure 2.7: Basic procedure of API 20E

2.5.2 Molecular analyses of selected isolates

2.5.2.1 DNA extraction and Polymerase Chain Reaction

DNA extraction was done using boil template methods (Medici *et al*, 2003). 1.5 ml micro-centrifuge tube was used to fill one milliliter of pre-enriched sample (inoculated and non inoculated). The cell was centrifuged at 14,000×g (10minutes).Supernatant was discarded and pellet was re-suspended with 300µl of DNase-RNase free distilled water (Sigma, Sigma–Aldrich, Gallarate, Milan, Italy) by vortexing. The tube was centrifuged at 14,000×g (5 minutes), and the supernatant was discarded carefully. The pellet was re-suspended in 200 µl of DNase-RNase-free distilled water (Sigma) by vortexing. The micro-centrifuge tube was incubated for 15 min at 100 °C and immediately chilled on ice. The tube was centrifuged for 5 min at 14,000×gat 4 °C. The supernatant was carefully transferred to a new micro-centrifuge tube and incubated again for 10 min at 100 °C and chilled immediately on ice. An aliquot of 5 µl of the supernatant was used as the template DNA in the PCR.

2.5.2.2 Choice of primers and optimization of primer concentration

Molecular identification of *E. coli* was done by amplification of the *uidA* gene that encodes for β-D-glucuronidase using primers that are specific (Table 2.2) for this gene in *E. coli*.UAL-1939 and UAR-2105 were used to amplify a 0.166 kb region of the *uidA* gene. Primer UAL-1939 was located between 1939 and 1952 bp and primer UAR-2105 was located between 2085 and 2104 bp closer to the carboxyl region of the *uidA* gene of *E. coli.*

Table 2.2: PCR oligonucleotide primers used to detect *E. coli*

Bacterium	Gene target	Amplicon Size (bp)	Primer	Sequence	Reference
Escherichia coli	*uidA*	166	UAL-1939 UAR-2105	TATGGAATTTCGCCG ATTTT TGTTTGCCTCCCTGCT GCGG	Bej *et al*, 2003

2.5.2.3 PCR analysis and detection of *uidA* gene

PCR amplification of *uidA* gene was carried out in a thermal cycler (applied biosystems, Singapore) using 200 µl of PCR tubes with a reaction mixture of 12.5µl. Reaction mixture was prepared using 1 µg template DNA, 2.5µl 2× Dream Taq™ PCR master mix (10× Dream Taq™ buffer, 2 µM dNTP mix and 1.25 U Dream Taq™ polymerase) and a 10 µM concentration of each PCR primer (Eurogentec S.A., Belgium) and was made up to 12.5 µL with ultra-pure

nuclease-free water. The following cycling parameters were used: denaturation of template DNA at 94 °C for 2 min, followed by 25 cycles of denaturation at 94 °C for 1 min, annealing of template DNA at 55 °C for 1 min and an extension time of 1 min at 72 °C for the primers. After the last cycle the samples were kept at 72 °C for 2 min to complete the synthesis of all strands (Momba *et al*, 2006; Tsai *et al.*,1993).

The amplified products were examined by running the PCR products on 1.5% agarose gel in 1× TAE buffer (pH 8.2). 10 µl of PCR products were mixed with 2 µl of gel loading dye and loaded into the well of the gel with the help of micropipette. Electrophoresis was done in horizontal electrophoretic apparatus using 1× TAE buffer at 65-70 volts until the dye reached to the end of the gel. The gel was stained with staining solution for 30 minutes and destained in distilled water for 15 minutes. The gel was observed under UV light in a gel documentation system (BIO-RAD, Italy), PCR products' sizes were estimated using the 100 base pair marker. Positive control includes reference strain of *Escherichia coli* of concentration of 2.5µl and negative control includes all the PCR reagent, except for the template DNA.

2.6 Storage of control and treated water samples

The control and treated water samples were stored for 6 months at room temperature (26-28 ^{0}C), and periodic quality parameters were analyzed. Each experiment was done in triplicate and the average results were presented in different tables and figures in result section.

2.7 Toxicity test for moringa seed powder and scallop powder

Cytotoxic effect of moringa powder and scallop powder was done on BHK and HeLa cell using tissue culture methods at the cell culture laboratory of the Centre for Advanced Research in Sciences, University of Dhaka. In brief, HeLa, a human cervical carcinoma cell line and BHK-21, a baby hamster kidney fibroblast cell line, was maintained in DMEM (Dulbecco's Modified Eagles medium; Sigma, UK) containing 1% penicillin streptomycin (1:1) and 0.2% gentamycin and 10% fetal bovine Serum (FBS). HeLa (1.4×10^{4}/100 µl) and BHK-21 (8×10^{4}/100 µl) cells were seeded onto 96-well plates and incubated at 37 °C + 5% CO_2 chamber. Cytotoxicity was examined under an inverted light microscope (Optika-XDS: 2ERGO, Italy) after 24hrs of incubation. Duplicate wells were used for each sample.

2.8 Pyrogen detection test using LAL test (gel clot method)

Tests were performed according to USFDA (2012) instructions.

2.8.1 Preparation of *E. coli* Control Standard Endotoxin (CSE)

All the reagents were equilibrated to room temperature prior to use.

Note: Plastic tubes are not recommended for making endotoxin dilutions.

A. The vial of endotoxin was reconstituted with 1.0 ml LAL Reagent Water. Now reconstituted CSE Potency is 10 EU/ml.

B. The vial of endotoxin was vortexed for at least 15 minutes.

C. The endotoxin was diluted with LAL Reagent Water to a concentration of 1EU/ml. This is accomplished by diluting the reconstituted endotoxin to 1/X, where X is the CSE potency in EU/ml as specified on the Certificate of Analysis. Using X as defined above, the general formula is 0.1 ml reconstituted endotoxin diluted with 0.1 (X-1) ml LAL Reagent Water. Example for X = 10 EU/ml: 0.1 ml of endotoxin was diluted with 0.1 (10-1) = 0.9 ml LAL Reagent Water.60 seconds vortexing was done before proceeding.

D. Using the 1 EU/ml endotoxin solutions, a serial two-fold dilution series (Table 2.3) were prepared that brackets the sensitivity of lysate as shown in the following example. Each dilution was vortexed for 60 seconds prior to proceed to the next dilution.

Table 2.3: Dilution series for use with lysate of 0.125 EU/ml sensitivity

Tube number	Water (ml)	Volume Added to Water	Endotoxin concentration (EU/ml)
1	1.0	1.0 ml from 1 EU/ml	0.5
2	1.0	1.0 ml from Tube 1	0.25
3	1.0	1.0 ml from Tube 2	0.125
4	1.0	1.0 ml from Tube 3	0.06
5	1.0	1.0 ml from Tube 4	0.03

2.8.2 Sample test procedure and methodology

Each assay included serial two-fold dilutions of the Control Standard Endotoxin (CSE) which brackets the labeled lysate sensitivity, dilutions of the test sample, and LAL Reagent Water to serve as a negative control. To avoid microbial or endotoxin contamination, 0.10 ml of standard, sample water were transferred carefully into the appropriate 10 x 75 mm reaction tube. 0.10 ml of the reconstituted lysate was added to each tube beginning with the blank then moving from lowest to highest concentration of endotoxin. Immediately following the addition of the lysate to

each tube, the contents were mixed thoroughly and the tube placed in a 37 °C ± 1 °C non-circulating hot water or dry heat bath. This procedure was followed for each dilution of the endotoxin. The unknown test sample must be run in parallel with the control standard endotoxin. The assay was done either as a yes/no test at a single dilution or as a quantitative test via a dilution series. The incubation time was determined from the time each tube is placed in the 37 °C ± 1 °C bath. Assay tubes should not be removed from incubation or disturbed prior to the time specified for reading the test. After 60 minutes (±2 minutes) of incubation, each tube was carefully removed and inverted 180 °C.

1. A positive reaction was characterized by the formation of a firm gel that remains intact momentarily when the tube is inverted.
2. A negative reaction was characterized by the absence of a solid clot after inversion. Increased turbidity or viscous lysate were also considered as negative result.
3. The reaction in each tube was recorded in columns as either positive or negative.

2.8.3 Procedure of avoiding product inhibition situation

The LAL reaction is enzyme mediated and, as such, has an optimal pH range, and specific salt and divalent cation requirements. Occasionally, test samples may alter these optimal conditions to an extent that the lysate is rendered insensitive to endotoxin. Negative results with samples which inhibit the LAL test donot necessarily indicate the absence of endotoxin.

Initially, each type of samples has been screened for product inhibition. A series of two fold dilutions of endotoxin in LAL reagent water and a similar series of endotoxin dilutions using samples as diluents have been prepared. Then the standard assay has been prepared. Positive and negative results have been recorded after the incubation time.

3.0 Results

Surface waters are vital and vulnerable sweet water resources that are critical for the sustenance of all life. Rivers, ponds, and lakes are waterways of strategic importance across the world, providing main water resources for domestic, industrial, and agricultural purposes (Ngwenya, 2006). Physical and chemical parameters of surface water collected from different sources in this study revealed that the water is suitable for aquatic life, irrigation and domestic purposes without any form of treatment. However, bacteriological analysis results showed that the source pond water was grossly contaminated than lake and river water.

The total coliform count and *E. coli*, indicative of faecal pollution, has the highest prevalence than other pathogens including *Vibrio* spp. and *Salmonella* spp. present in the raw pond water samples. However, lower level of coliform and *E. coli* count were observed in lake and river water. No *Salmonella* spp. and *Vibrio* spp. was detected in any of the lake and river water analyzed. The treated water samples were stored for 6 months at room temperature (26-28 ^{0}C), and periodic quality parameters were analyzed to observe if resuscitation of injured bacteria was occured.

All the trials were replicated three times and reported data represented the mean values obtained from five individual trials, with each of these values obtained from duplicated samples. Data were subjected to statistical analysis using the Microsoft Excel Program 2007 (Redmond, Washington DC, USA). Significant differences in plate count data were established by the least significant difference at the 5% level of significance.

3.1 Cultural identification

Cultural characteristics of the bacteria isolated from different surface waters were observed carefully for presumptive identification (1-2mm pink colonies for *E. coli*; 1-2mm straw colonies for *E. coli* O157:H7; black coloured colonies for *Salmonella* spp; 2-3 mm yellow to blue-green colonies for *Vibrio* spp.) and then confirmed using API 20E tests. Appearance of bacterial colony on different culture media used in this study was in consideration (Figure 3.1). All the bar diagrams were plotted based on cultural and API tests.

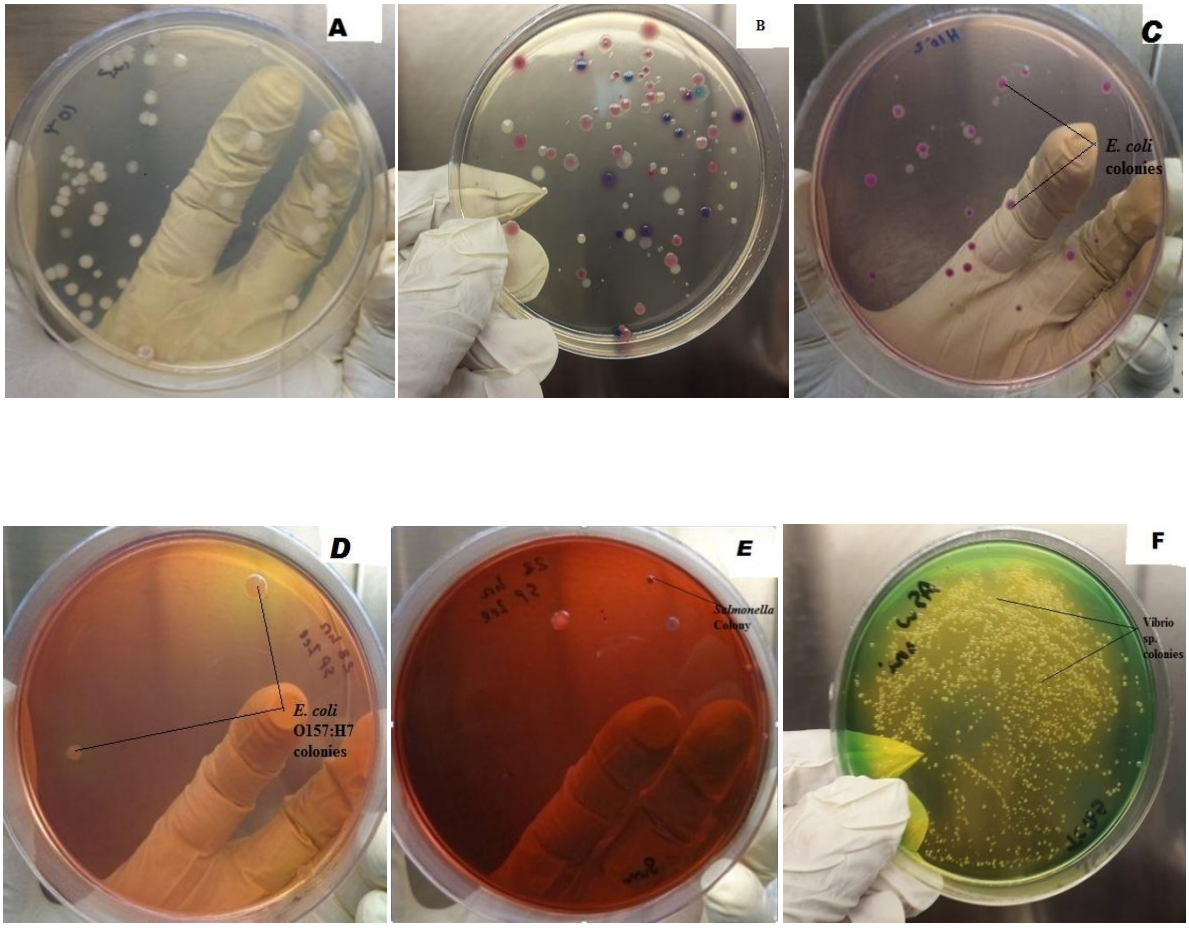


Figure 3.1: Representative culture plates (A- TSA, B- Chromocult, C- SMAC, D- CT-SMAC, E- XLD Agar, F- TCBS Agar) of primary bacterial isolates

3.2 Analytical Profile Index (API) test

After the cultural identification, API 20E (bioMerieux, France) tests were performed to confirm the identity of bacteria isolated from surface water (Figure 3.2 and 3.3).

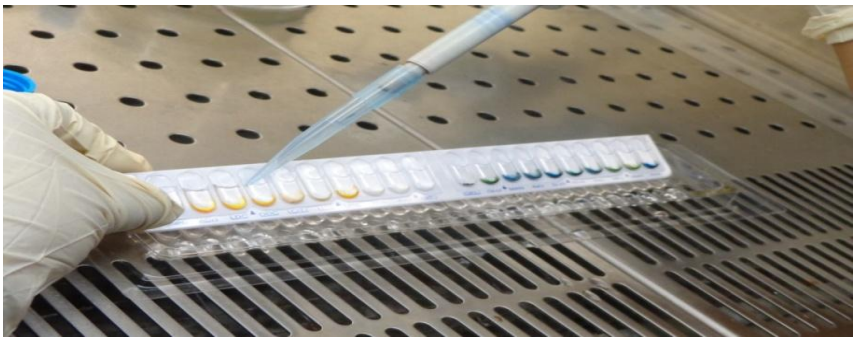


Figure 3.2: Inoculation of bacterial solution into API 20E kit

Figure 3.3: API 20E strips confirmed *E. coli*

Table 3.1: Interpretation table of API 20E test (adapted from Biomerieux.com)

Tests	Active Ingredients	Qty (mg/ cup)	Reactions/Enzymes	Results	
				Negative	Positive
ONPG	2-nitrophenyl βD-galactopyranoside	0.223	β galactosidase	Colorless	Yellow
ADH	L-arginine	1.9	Arginine Dihydrolase	Yellow	Red/Orange
LDC	L-Lysine	1.9	Lysine Decarboxylase	Yellow	Red/Orange
ODC	L-Ornithine	1.9	Ornithine Decarboxylase	Yellow	Red/Orange

CIT	Tri sodium citrate	0.756	Citrate Utilization	Pale green/ yellow	Blue-green/ blue
H_2S	Sodium Thiosulphate	0.075	H_2S production	Colorless/greyish	Black deposit/thin line
URE	Urea	0.76	Urease	Yellow	Red/Orange
TDA	L-tryptophan	0.38	Tryptophan deaminase	TDA /Immediate	
				Yellow	Reddish Brown
IND	L-tryptophan	0.19	Indole Production	James/ Immediate	
				Colorless Pale green/yellow	Pink
VP	Sodium Pyruvate	0.19	Acetoin Production (Voges Proskauer)	VP_1+VP_2 / 10 min	
				Colorless	Pink/red
Gel	Gelatin (Bovine origin)	0.6	Gelatinase	No difusion	Diffusion of black pigment
GLU	D-glucose	1.9	Fermentation/Oxidation (GLU)	Blue/Blue-Green	Yellow/Greyish yellow
MAN	D-Mannitol	1.9	Fermentation/Oxidation(MAN)	Blue/Blue-Green	Yellow
INO	Inositol	1.9	Fermentation/Oxidation (INO)	Blue/Blue-Green	Yellow
SOR	D-Sorbitol	1.9	Fermentation/Oxidation(SOR)	Blue/Blue-Green	Yellow
RHA	L-Rhamnose	1.9	Fermentation/Oxidation(RHA)	Blue/Blue-Green	Yellow
SAC	D-Sucrose	1.9	Fermentation/Oxidation(SAC)	Blue/Blue-Green	Yellow
MEL	D-Mellibiose	1.9	Fermentation/Oxidation(MEL)	Blue/Blue-Green	Yellow
AMY	Amygdaline	0.57	Fermentation/Oxidation(AMY)	Blue/Blue-Green	Yellow
ARA	L-arabinose	1.9	Fermentation/Oxidation (ARA)	Blue/Blue-Green	Yellow

3.3 Effectiveness of low-cost solar pasteurization device in reducing microbial population of surface water

3.3.1 Environmental water samples: Seven surface water sites were selected and water samples from these sites were used for treatment with solar pasteurization device. Among the surface water sample sites, there were three ponds (Shahidullah Hall, Jagannath Hall and Bangla Academy), three rivers (Shitalakshya, Padma, and Meghna), and a lake (Dhanmondi lake).

Mean values of microbial quality parameters of ponds, lakes and river water samples have been shown in the figure 3.4, 3.5 and 3.6.

Bacteriological analysis results showed that the source pond water was grossly contaminated compared to lake and river water. The total coliform count and *E. coli*, indicative of faecal pollution, recorded as for pond 4.0 and 3.8 log CFU/ml respectively. However, lower level of coliform and *E. coli* counts were observed in lake (3.9 and 3.6 Log CFU/ml respectively) and river (3.9 and 3.1 Log CFU/ml respectively) water. On an average, irrespective of sample source, substantial number of resident bacteria (for pond 4.7 Log CFU/ml, for lake 4.1 log CFU/ml and for river 4.5 log CFU/ml) was recorded in raw water (figure 3.4, 3.5, 3.6). Otherwise, *E. coli* O157:H7 was recorded as 3.3 log CFU/ml (pond sample), 3.5 log CFU/ml (lake water) and for river water sample, no *E. coli* O157:H7 was recorded. Presence of *Vibrio* spp. and *Salmonella* spp. were not found in any of the water samples analyzed.

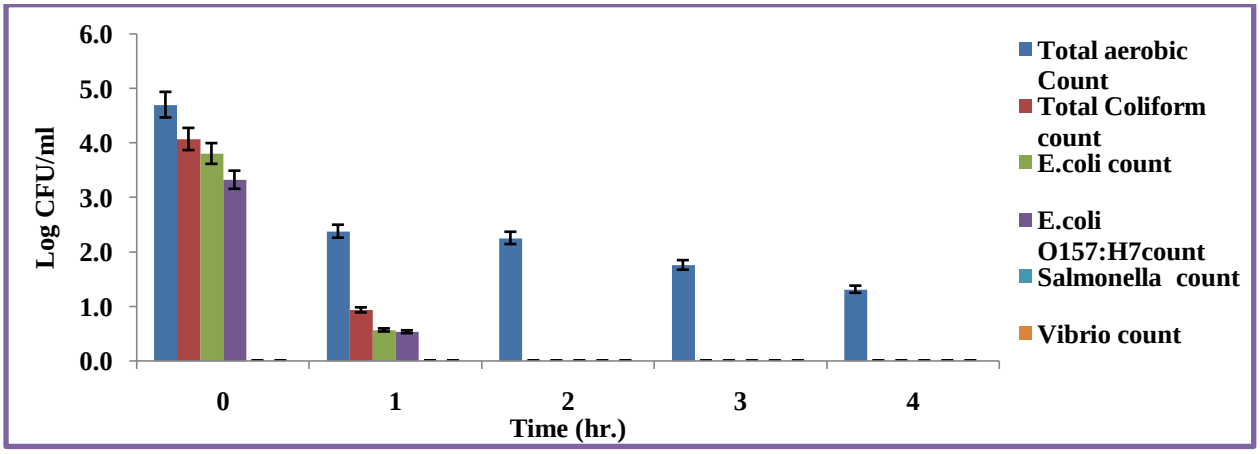


Figure 3.4: Effect of solar pasteurization device on total aerobic, total coliform, *E. coli*, *Salmonella* spp., *Vibrio* spp. count of pond water. In the control water sample, *Salmonella* spp. and *Vibrio* spp. were below the detection limit

The weather condition on the treatment days observed and recorded as clear, sunny, high humidity etc. Sunlight intensities were in the range of 1000 to 1200 watts per square meter on a horizontal surface. The initial day temperatures were recorded between 28-33 °C and starting

time of treatment process varies from 9.00 to 10.00 hour of local time. After treatment, the temperature reached to maximum 73-84 °C at the treatment point of water samples at 13.00 to 14.00 hour of local time.

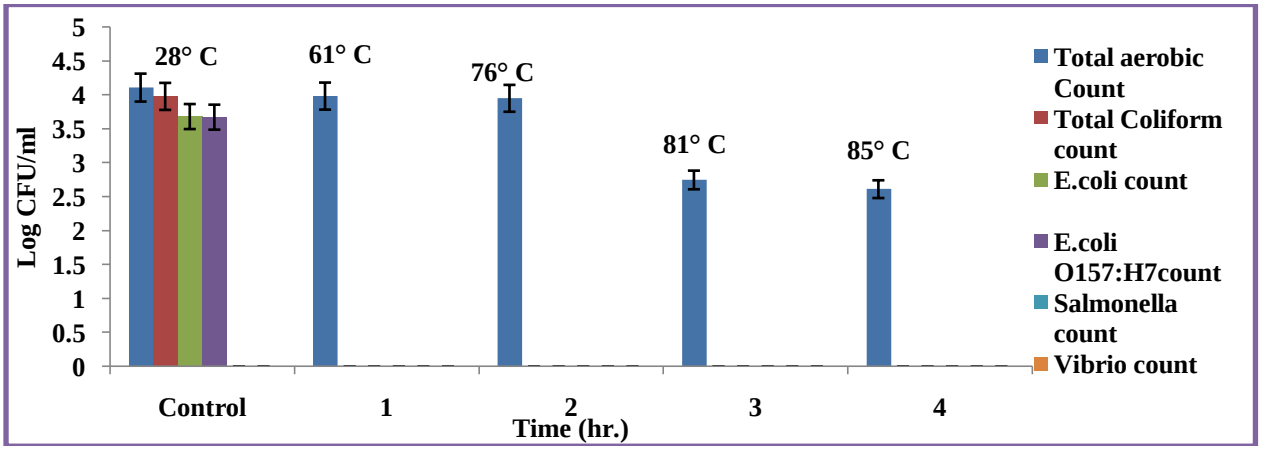


Figure 3.5: Effect of solar pasteurization device on total aerobic, total coliform, *E. coli*, *Salmonella* spp., *Vibrio* spp. count of Dhanmondi Lake water. In the control water sample, *Salmonella* spp. and *Vibrio* spp. were below the detection limit

As the temperature increased by the effect of solar pasteurization device, the initial aerobic count declined to 2.3 log CFU/ml (pond water sample), 4.0 log CFU/ml (lake water sample), 1.8 log CFU/ml (river water sample) after 1 hour of exposure and decreased further in 2 hour and 3 hour treatment and finally reached to 1.3 log CFU/ml (pond), 2.6 log CFU/ml (lake) and 0.4 log CFU/ml (river) at 4 hour of sunlight exposure. Total coliform, *E. coli* and *E. coli* O157:H7 population decreased significantly after 1 hour of exposure and no survivors were recorded after 2 hour of exposure and thereafter (Figure 3.4, 3.5, 3.6). As control samples, *Salmonella* and *Vibrio* spp. count were below the detection limit in the treated samples.

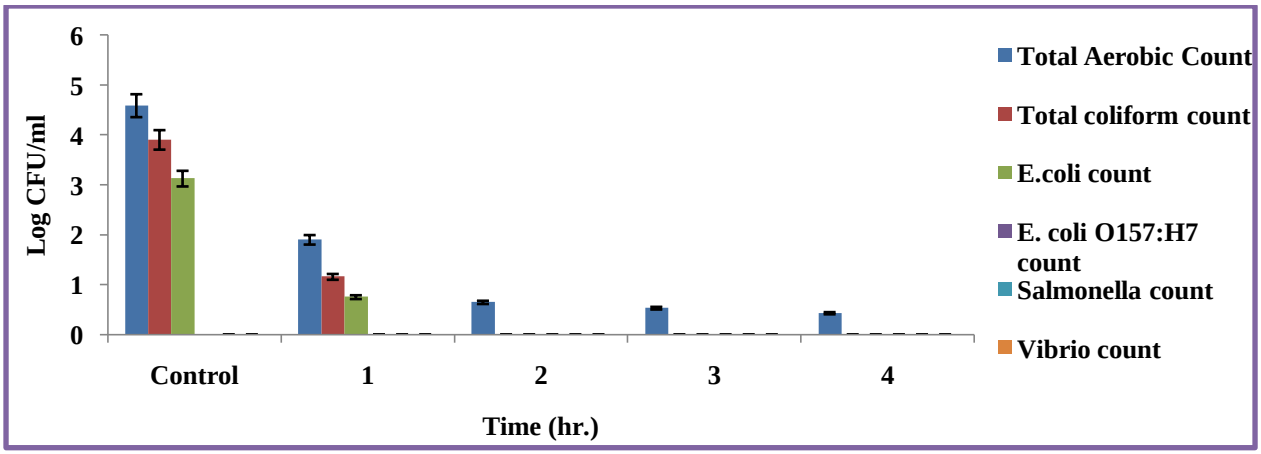


Figure 3.6: Effect of solar pasteurization device on total aerobic, total coliform, *E. coli*, *Salmonella* spp., *Vibrio* spp. count of river water. In the control water sample, *Salmonella* spp. and *Vibrio* spp. were below the detection limit

3.3.1.1 Shelf-life study of treated water: The control and treated water samples from seven environmental sources were stored for 6 months at room temperature (26-28^{0}C), where periodic analysis of quality determining microbial population (total aerobic bacteria, total coliform, *E. coli*, *E. coli* O157:H7, and *Salmonella* spp.) were done. Results are represented through bar diagram in figure 3.7, 3.8, 3.9 for pond, lake and river water samples, respectively. Irrespective of sample source, none of the bacteria was detected throughout the study (Figure 3.7, 3.8, 3.9). This finding suggested that, the methodology used in this study could be useful for household level use.

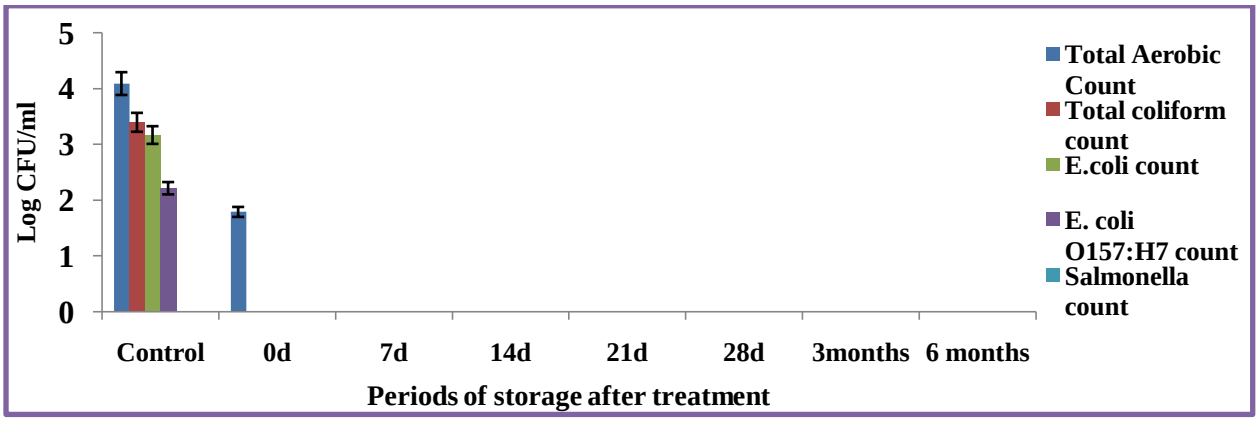


Figure 3.7: Mean value of bacteriological quality of 6 months stored treated pond water at ambient temperature

In case of all three types of sample water, presence of pathogens was being noticed before treatment.

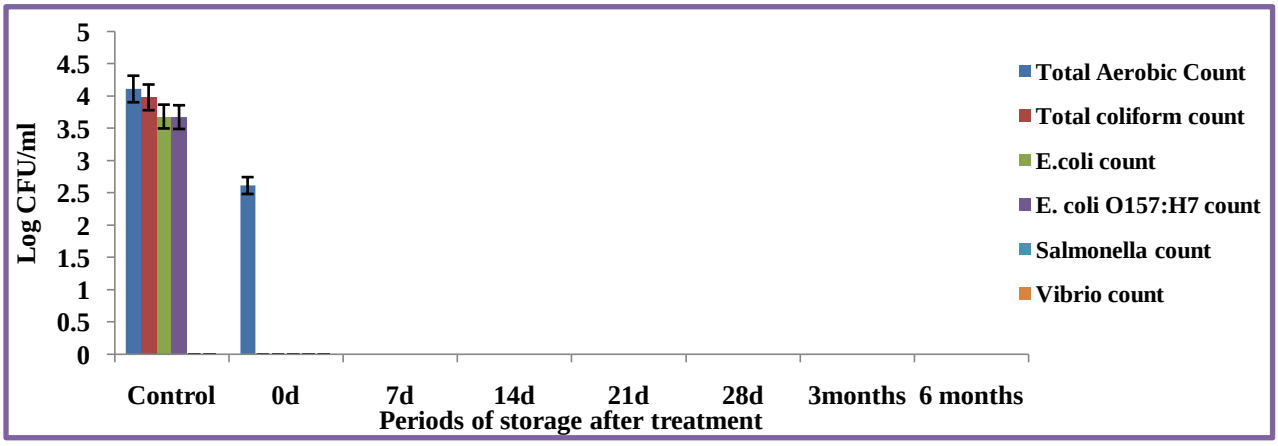


Figure 3.8: Bacteriological quality of 6 months stored treated Dhanmondi Lake water at ambient temperature

Different level of microbial reduction was observed varying on sample types just after the treatment and it seemed to be very effective for river water samples to bring significant reduction at the initial day of storage study. For all the sample types, results showed that, our applied treatment was able to halt the incidence of microbial resuscitation/growth, thus no bacterial cell came into our culture based microbial isolation technique throughout the end of storage study.

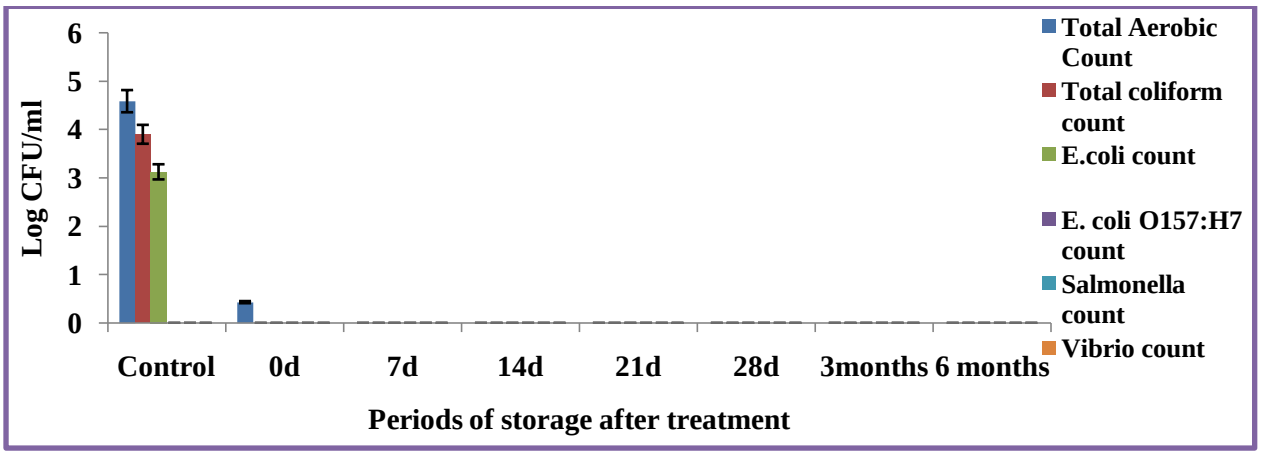


Figure 3.9: Bacteriological quality of 6 months stored treated river water at ambient temperature

3.3.2 Spiked study experiment: Intentional application of known number of microorganisms in test samples is called ‘‘spiked/inoculation study’’. The results evidenced the actual number of reduction of microorganisms in any test sample. Rifampicin resistant *E. coli* (marker bacteria) is not temperature resistant rather than antibiotic resistant bacteria, so solar radiation has an effect to destroy it. Based on the theory, the spiked study has been experimented. All the bar diagrams of the innovated techniques were based on the cultural results.

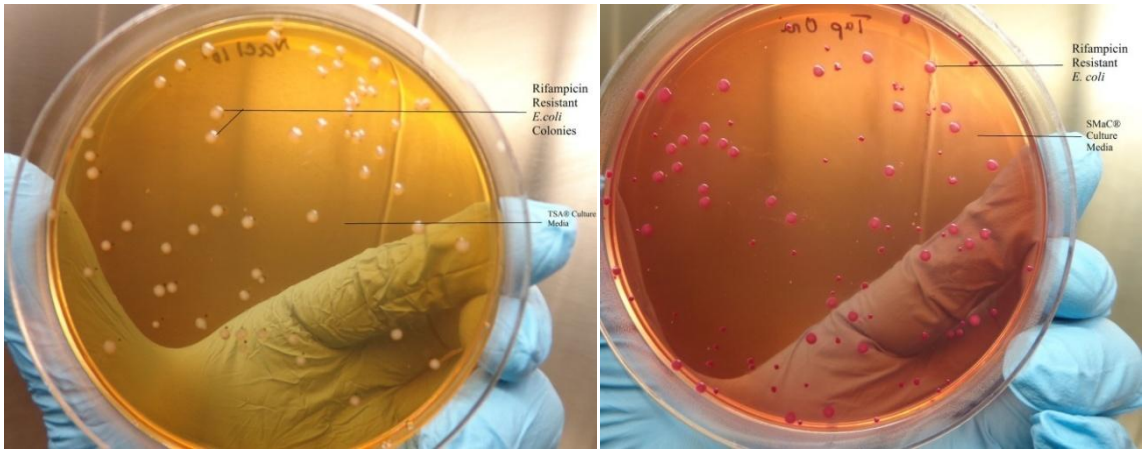


Figure 3.10: Representative culture plates of spiked study

3.3.2.1 Result of the experiment day of spiked samples: Mean values of the survivability of rifampicin resistant *E. coli* of three ponds have been shown in the figure 3.11. After 1 hour treatment, reduction of *E. coli* was very significant on both TSAR and SMACR and after 2 hours treatment, presence of rifampicin resistant *E. coli* cells was found to stay below the detection limit.

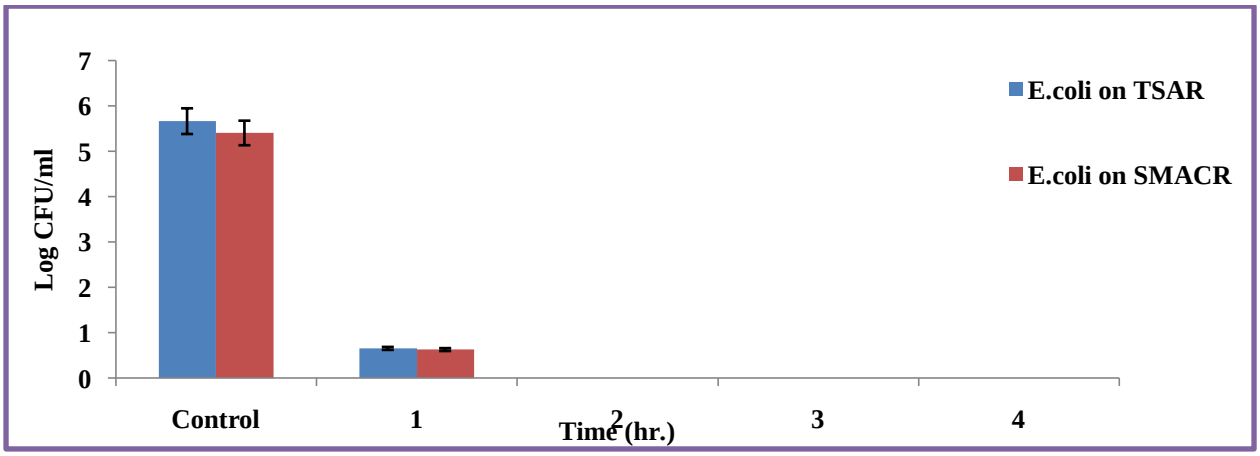


Figure 3.11: Effect of Solar pasteurization device on *E. coli* count (TSAR and SMACR) of rifampicin resistant bacteria (marker bacteria) of pond water

According to figure 3.12, survival of rifampicin resistantmarker bacteriaof Dhanmondi Lake was not noticed when the pasteurization temperature was as high as 71 ^{0}C (4th hour) even as low as 49^{o}C (1st hour) on both TSAR and SMACR mediadue to the heating effect of solar pasteurization device.

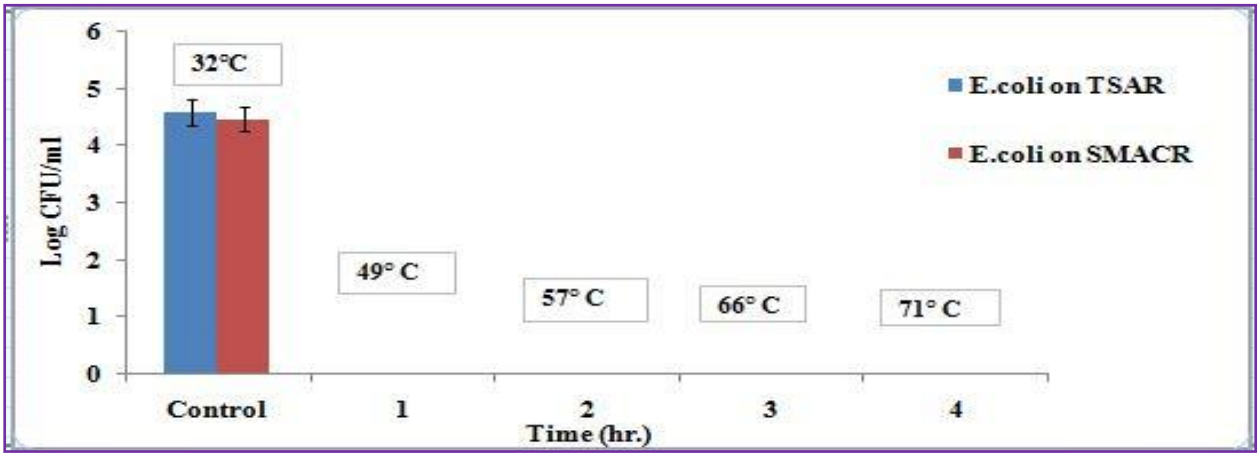


Figure 3.12: Effect of solar pasteurization device on *E. coli* count (TSAR and SMACR) of rifampicin resistant bacteria (marker bacteria) of Dhanmondi Lake water

Mean values of survivability of rifampicin resistant *E. coli* of three rivers have been showed in the figure 3.13. After 1 hour of treatment, survival of rifampicin resistant *E. coli* was found below the detection limit and thereafter. It indicates that, no resuscitation of injured bacteria occured after the treatment.

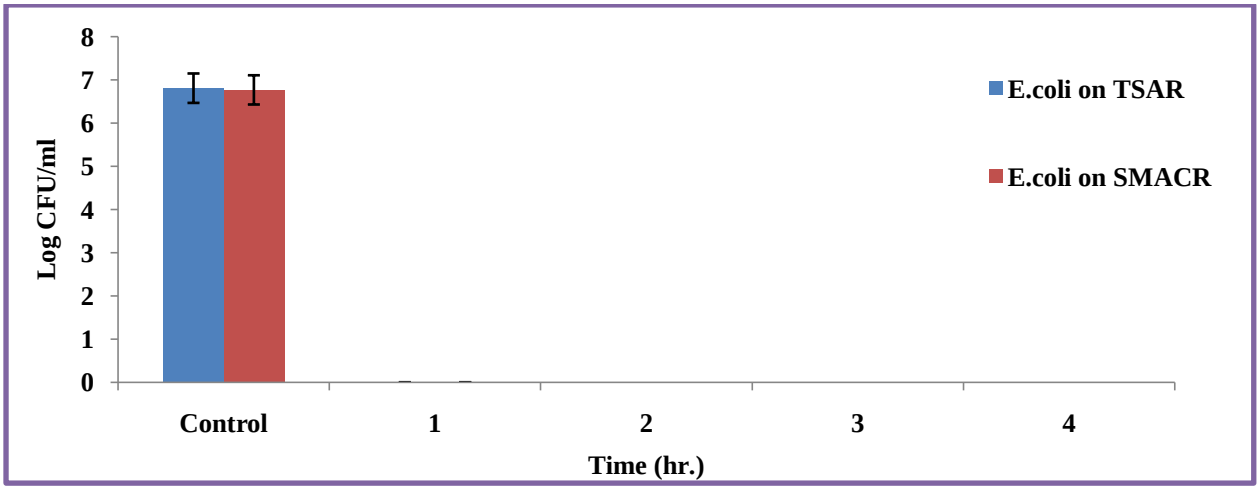


Figure 3.13: Effect of solar pasteurization device on*E. coli* count (TSAR and SMACR) of rifampicin resistant bacteria (marker bacteria) of river water

3.3.2.2 Shelf-life study of spiked samples: Survivability of rifampicin resistant *E. coli* were analyzed after storing the control and treated of seven inoculated water samples for 6 months at room temperature (26-28 °C). Treated water samples were divided into seven different sterile container to maintain strict asepticity of the sample and periodically analysis was performed. Results are represented through bar diagram in figure 3.14, 3.15, 3.16 for pond, lake and river water samples respectively.

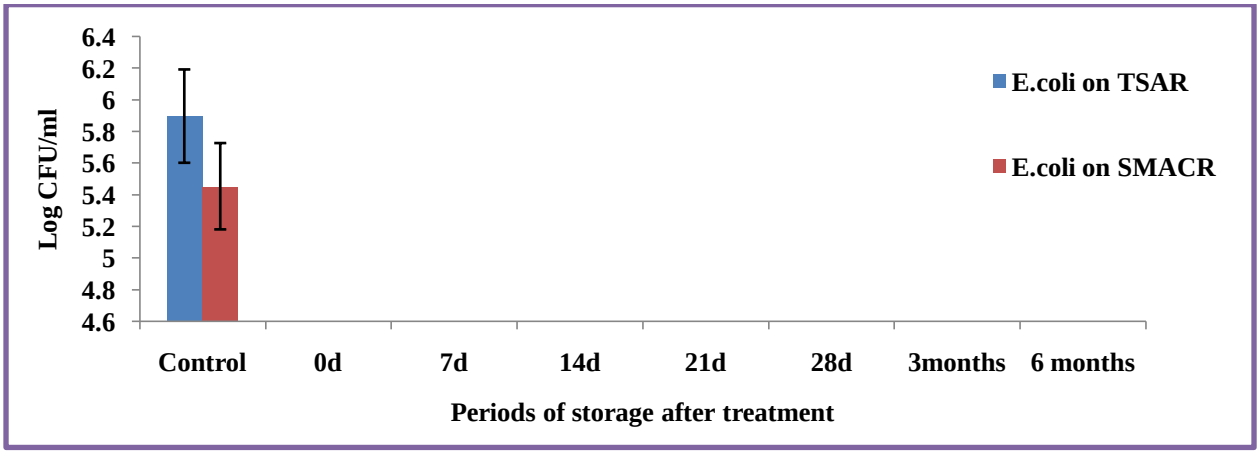


Figure 3.14: Bacterial (spiked) quality of 6 months stored treated pond water at ambient temperature

In case of all three types of sample water, presence of marker bacteria was being noticed before treatment as 5.9 and 5.4 log CFU/ml (pond spiked sample), 4.5 and 4.4 log CFU/ml (lake spiked sample), 6.6 log CFU/ml (river spiked sample) on TSAR and SMACR respectively.

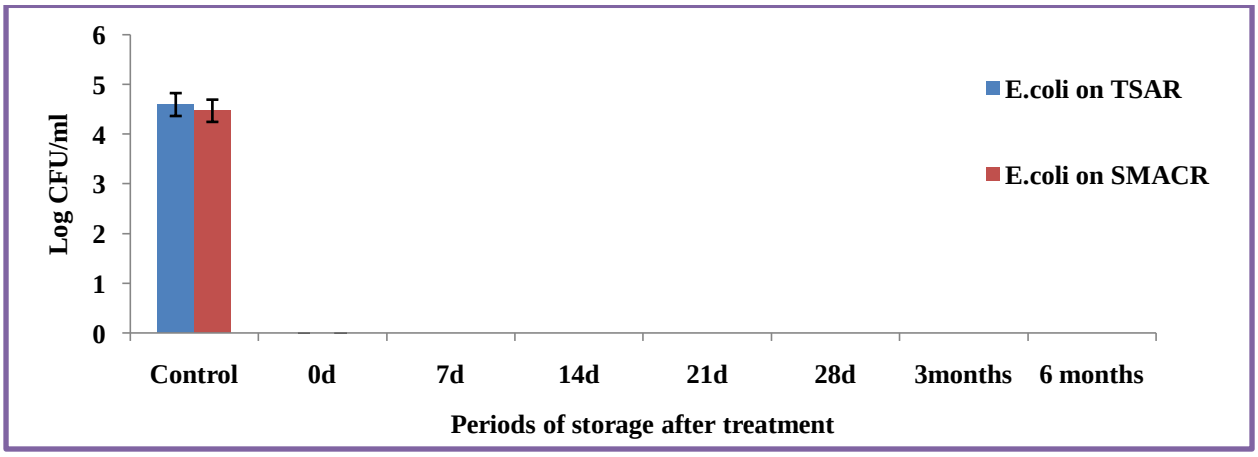


Figure 3.15: Bacterial (spiked) quality of 6 months stored treated Dhanmondi Lake water at ambient temperature

For all the sample types, results showed that, our applied treatment was able to cease the rifampicin resistant *E. coli* resuscitation, thus no bacterial cell came into detection limit throughout the end of storage study.

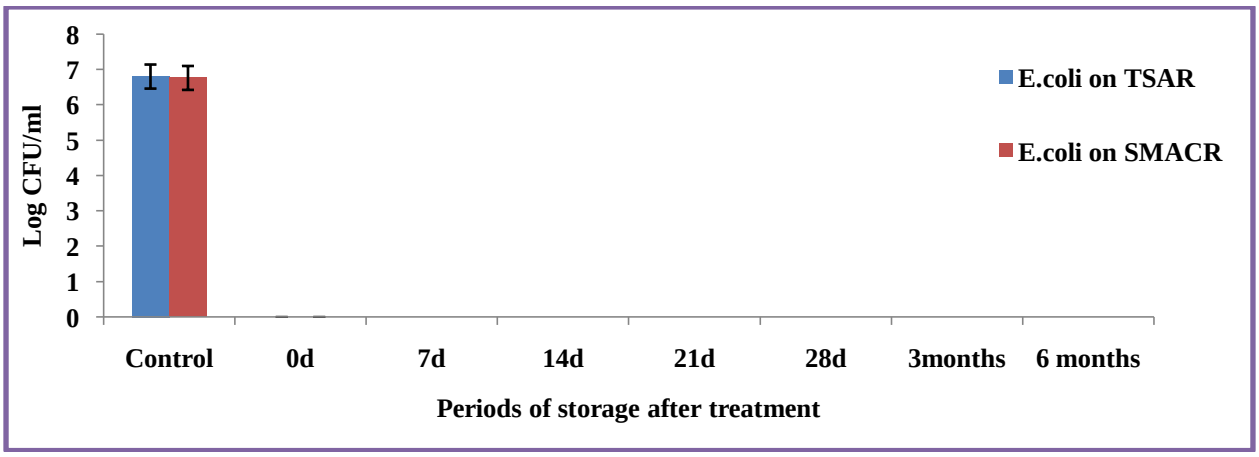


Figure 3.16: Bacterial (spiked) quality of 6 months stored treated river water at ambient temperature

3.3.3 Physico-chemical parameters of control and treated water samples:

To know the physico-chemical status of control and treated water samples of seven experimental sites, different tests have been conducted, the average data of seven sampling sites are also compared with EPA and BDS standard (Table 3.1).

All the parameters including color, odor, taste, pH, salinity, turbidity, conductivity, BOD, COD and different types of metals like iron, manganese, zinc etc were within the USEPA standard and BDS standard after treatment process. The environmental control samples have lower concentration of Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) and the natural minerals level (Iron, Manganese, Zinc) was also in very low amount.

Table 3.2: Physico-chemical Parameters of treated and untreated samples along with USEPA and BSTI Standard (treated with solar pasteurization device)

Parameters	Mean value of control samples	Mean value of treated samples	US EPA standard for drinking water	BDS standard
Color	Gray to yellowish (16-19 color unit)	15 (color unit)	15 (color unit)	5 (Haxen unit)
Odor	Odorous	Odorless	3 threshold odor number	Unobjectionable
Taste	Fishy-sour smelly	Agreeable	Agreeable	Agreeable
pH	6.9-7.7	7.1-7.6	6.5-8.5	6.4-7.4
Salinity (ppt)	0.1	0.1	0.1	0.1
Turbidity (NTU)	10.9-14.4	0.9-3.8	0.5-1.00	5.0
TDS	302-346	310-345	500	500
Conductivity µs/cm	600-780	620-690	NS	NS
BOD (mg/l)	2.1-2.5	2.71-2.76	5.0	5.0
COD (mg/l)	2.214-3.195	2.27-2.31	40	40
Iron (mg/L)	0.04	0.04	0.3	0.30
Manganese (mg/L)	0.02	0.02	0.05	0.50
Zinc (mg/L)	0.01	0.01	5.00	3.00
Lead (mg/L)	0.000	0.0004	0.015	0.01
Arsenic (mg/L)	0.002-0.003	0.002	0.01	0.05
Cadmium (mg/L)	0.00	0.00	0.005	0.003

*NS: Not specified

3.3.4 Detection of *uid A* gene by DNA extraction, PCR and Agarose gel electrophoresis (treated with solar pasteurization device)

Detection of *uidA* gene of *E. coli* was performed with DNA extraction with boiled template method (Medici *et al*, 2003), followed by PCR and 1.5% agarose gel electrophoresis. All the

control samples gave the specific band respective to marker (166bp), while treated samples were negative respective to *uidA* gene marker (figure 3.17).

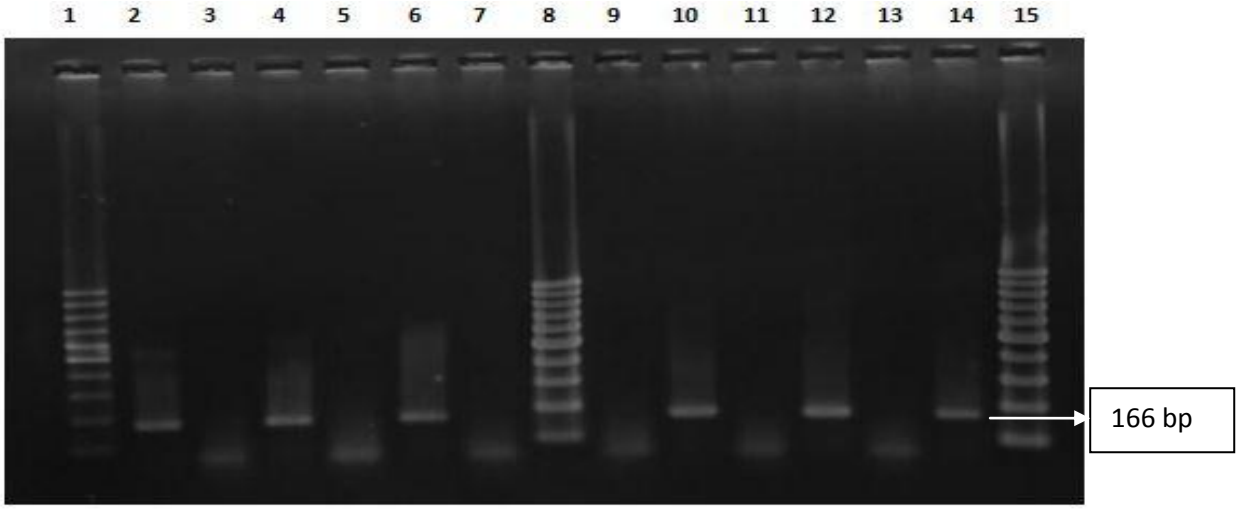


Figure 3.17: Representative *E. coli* was confirmed by detecting specific *uidA* gene using molecular PCR method. Lanes: 1,8 and 15 represents 100-bp standard DNA ladder; Lane 2, 4, 6, 10, 12 represents non-treated samples; Lane 3, 5, 7, 9, 11 represents treated samples; Lane 13 negative control; & Lane 14 positive control

3.4 Effectiveness of combined moringa seed powder and scallop powder followed by bio-sand (sand-charcoal-gravel) filter in the purification of surface water

3.4.1 Environmental water samples: Seven surface water sites were selected as solar pasteurization treatment and water samples from these sites were used for treatment with moringa seed powder, scallop powder along with sand-charcoal-gravel filter.

Mean values of microbial quality parameters of three ponds have been shown in the figure 3.18.After treatment, total aerobic count were (In control samples 5.8 log CFU/ml) reduced significantly(1.0 log CFU/ml after treatment) and other microbial quality parameters were below the detection limit.

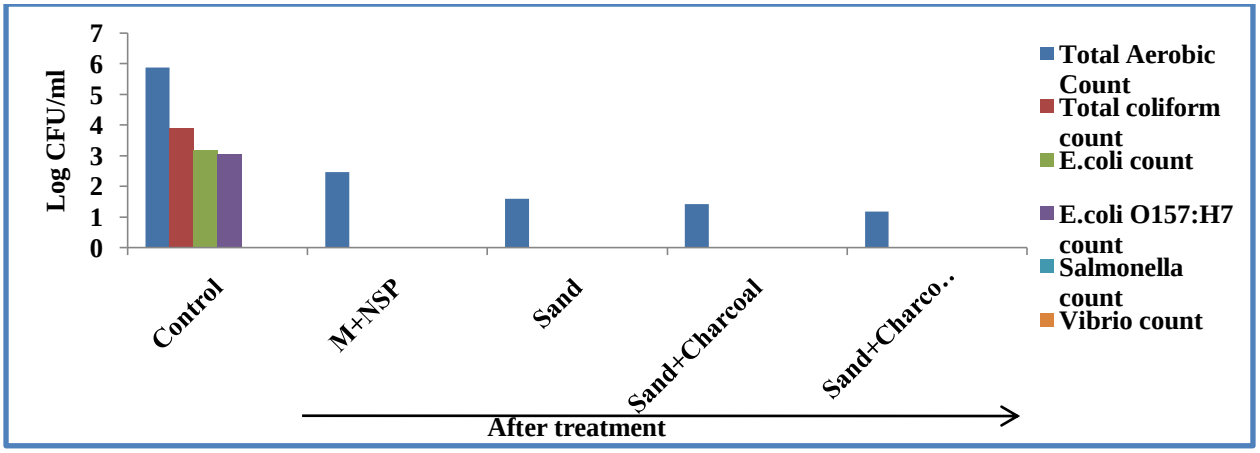


Figure 3.18: Effect of moringa seed powder, scallop powder and sand-charcoal-gravel filter on total aerobic, total coliform, *E. coli*, *Salmonella* spp., *Vibrio* spp. count of pond water. In the control water sample, *Salmonella* spp and *Vibrio* spp., were below the detection limit

The reason behind the separation of lake water from ponds and rivers is because of slow-moving body of water surrounded by land. Figure 3.19 represents the presence of pathogenic and indicator bacterial population remained below the detection limit after moringa and scallop powder treatment and thereafter.

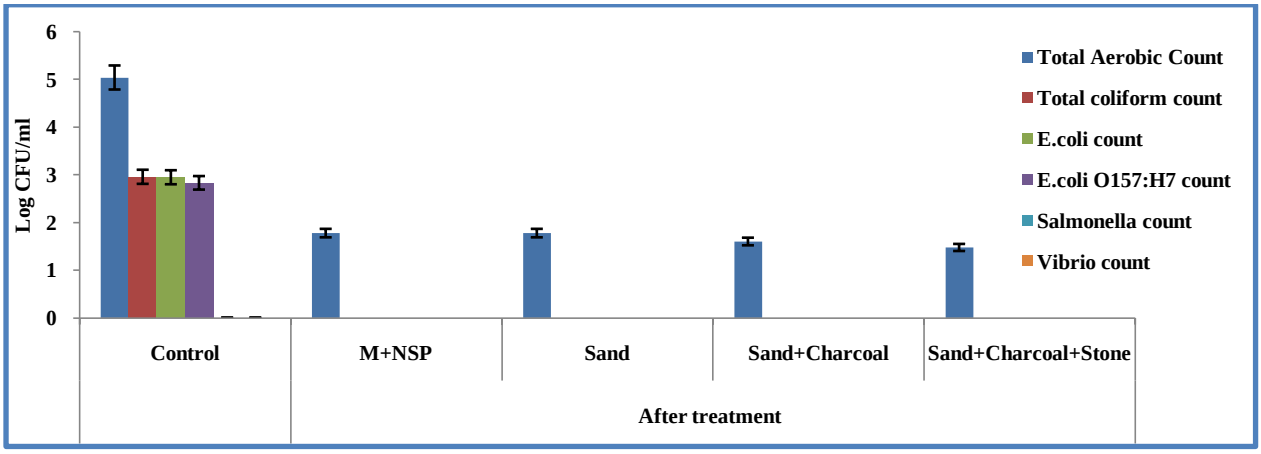


Figure 3.19: Effect of moringa seed powder, scallop powder and sand-charcoal-gravel filter on total aerobic, total coliform, *E. coli*, *Salmonella* spp., *Vibrio* spp. count on Dhanmondi Lake water. In the control water sample, *Salmonella* spp. and *Vibrio* spp., were below the detection limit

In case of three river's water, from figure 3.20 that, after moringa and scallop powder treatment total aerobic bacterial count was reduced significantly. Otherwise, elimination of total coliform count, *E. coli* and *E. coli* O157:H7 count was observed after moringa and scallop treatment followed by bio-sand filtration. All the trials were replicated three times and reported data

represented the mean values obtained from five individual trials, with each of these values obtained from duplicated samples. Most of the samples have lower amount of coliform *E. coli*. *Vibrio* spp. were not found in the pond, lake and river samples. After the treatment, coliform and *E. coli* were most sensitive to the treatment and after the treatment period, they were below the detection limit (Detection limit <1 CFU/ml)

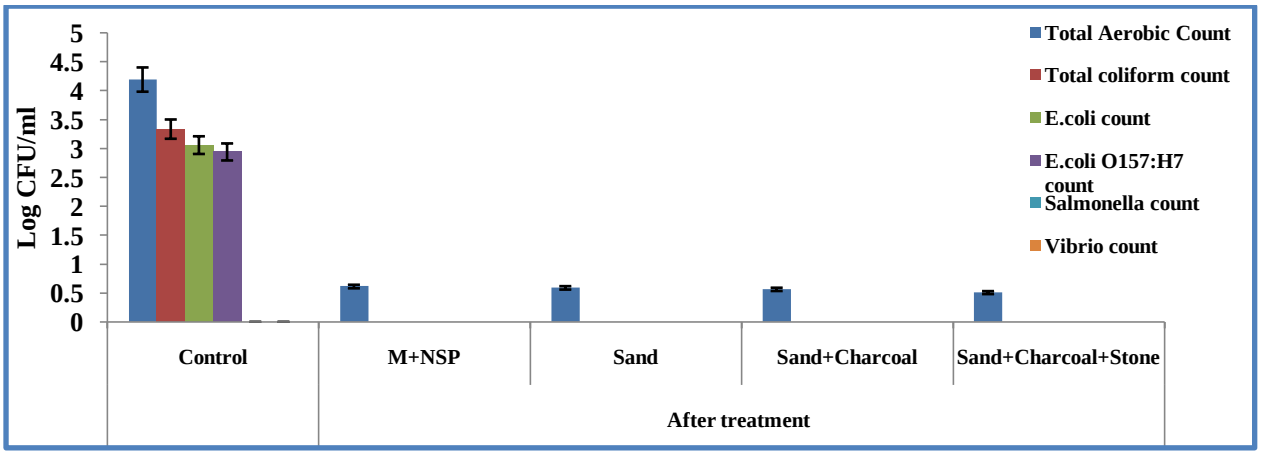


Figure 3.20: Effect of moringa seed powder, scallop powder and sand-charcoal-gravel filter on total aerobic, total coliform, *E. coli*, *Salmonella* spp., *Vibrio* spp. count on river water. In the control water sample, *Salmonella* spp. and *Vibrio* spp., were below the detection limit

3.4.1.1Storage study of control and treated environmental samples

Ambient temperature (26-28 °C) was maintained to preserve the control and treated water samples from seven environmental sources for 6 months and periodic analysis of quality determining microbial population (total aerobic bacteria, total coliform, *E. coli*, *E. coli* O157:H7, and *Salmonella* spp.) were performed. Results are represented through bar diagram in figure3.21, 3.22, 3.23 for pond, lake and river water samples respectively.

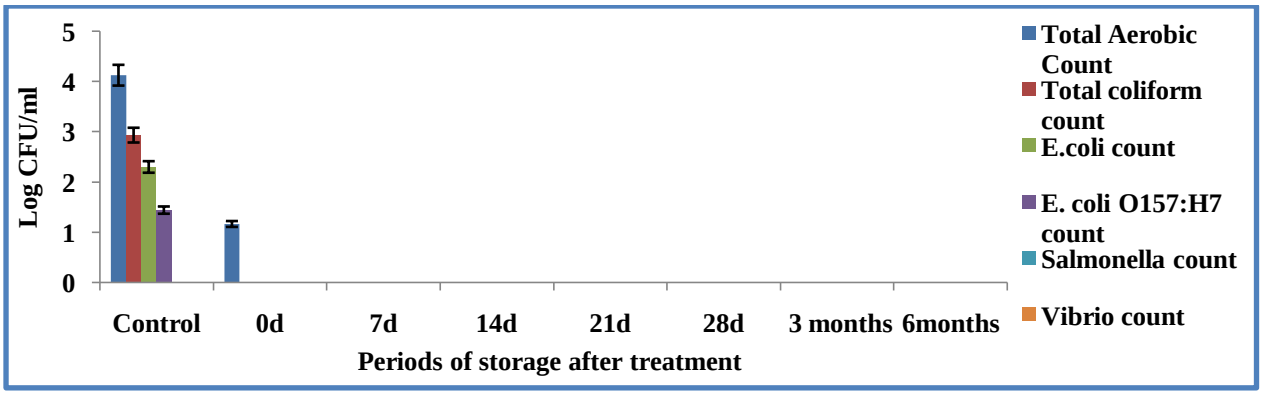


Figure 3.21: Bacterial quality of 6 months stored treated pond water at ambient temperature

Presence of the pathogens profile was observed before treatment in all three types of sample water. Different level of microbial reduction was observed varying on sample types just after the treatment.

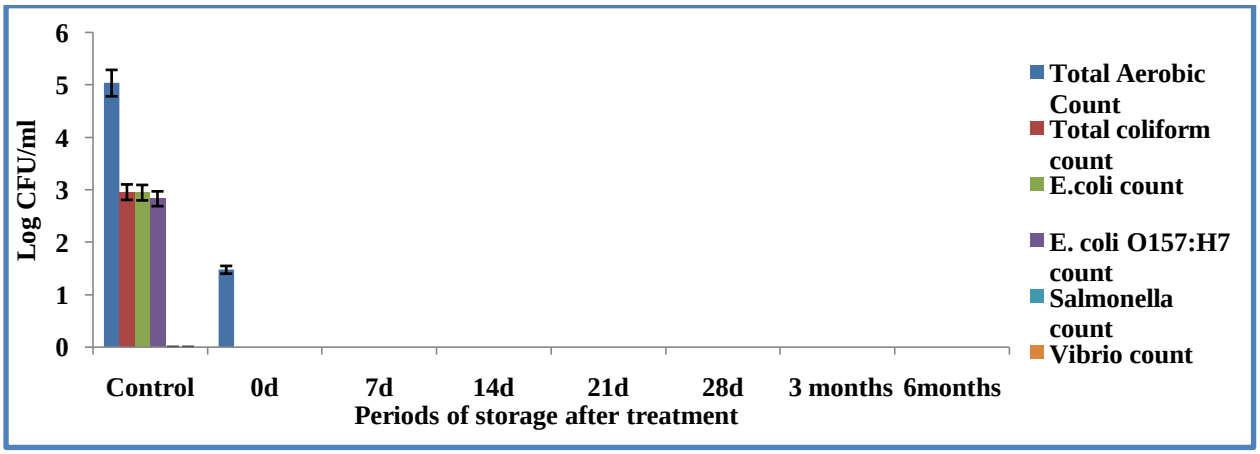


Figure 3.22: Bacterial quality of 6 months stored treated Dhanmondi Lake water at ambient temperature

For all the sample types, results showed that, the applied treatment succeeded to halt the incidence of microbial resuscitation/growth, thus no bacterial cell came into our culture based microbial isolation technique throughout the storage study.

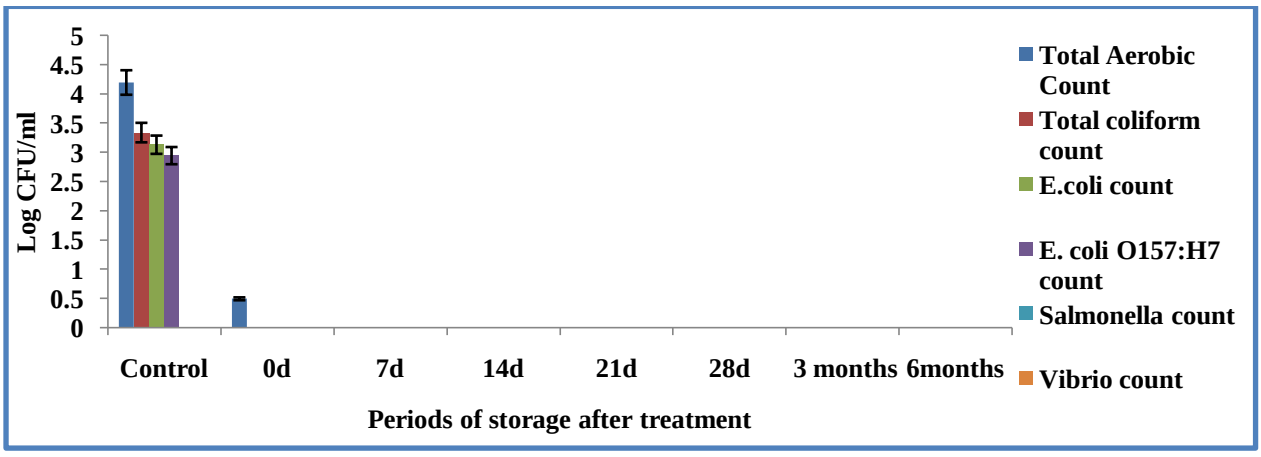


Figure 3.23: Bacterial quality of 6 months stored treated river water at ambient temperature

3.4.2 Spiked study: Rifampicin resistant *E. coli* was used here as marker bacteria to observe the efficiency of moringa seed powder, scallop powder and bio-sand filtration. Rifampicin is a lavishly used (5 µg/ml) antibiotic and rifampicin resistant *E. coli* is available in the

environmental water samples. But in this study, 10 times concentrated rifampicin (50µg/ml) was used to make *E. coli* resistant.

Plating on media containing rifampicin greatly minimized the interference of naturally occurring microorganisms and facilitate the detection of test pathogen on recovery media.

Mean values of the survivability of rifampicin resistant *E. coli* have been shown in the figure 3.24, 3.25 and 3.26 for pond, lake and river water samples respectively.

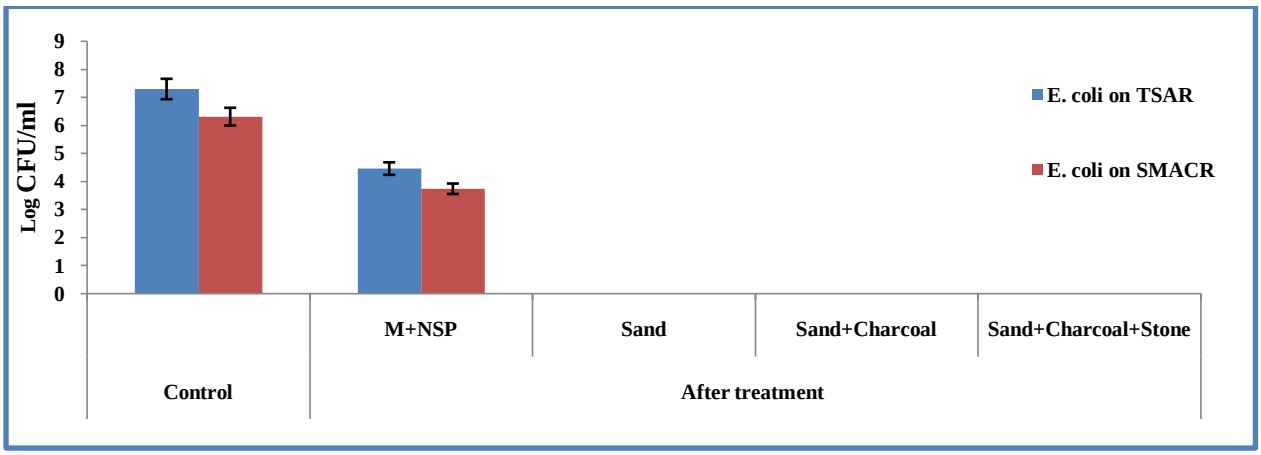


Figure 3.24: Effect of moringa seed powder, scallop powder and sand-charcoal-gravel filter on *E. coli* count (TSAR and SMACR) of rifampicin resistant bacteria (marker bacteria) of pond water

Here, Tryptic Soy Agar with Rifampicin (TSAR) and Sorbitol MacConkey Agar with Rifampicin (SMACR) were used for culture dependent technique.

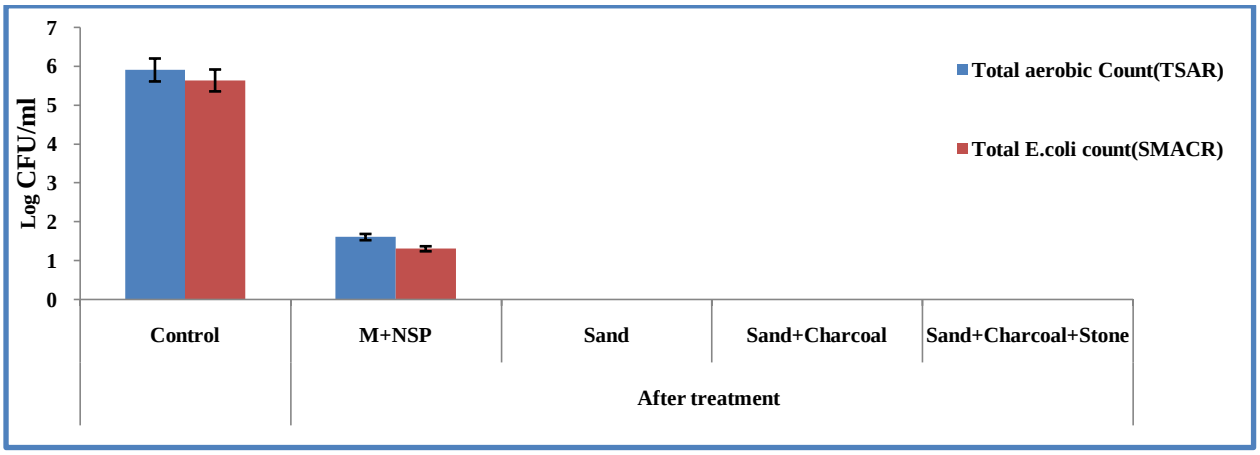


Figure 3.25: Effect of moringa seed powder, scallop powder and sand-charcoal-gravel filter on *E. coli* count (TSAR and SMACR) of rifampicin resistant bacteria (marker bacteria) of Dhanmondi Lake water

Irrespective of sample types and sources, after moringa and scallop powder treatment, reduction of *E. coli* was very significant on both TSAR and SMACR and after bio-sand treatment, survival of rifampicin resistant *E. coli* was found below the detection limit.

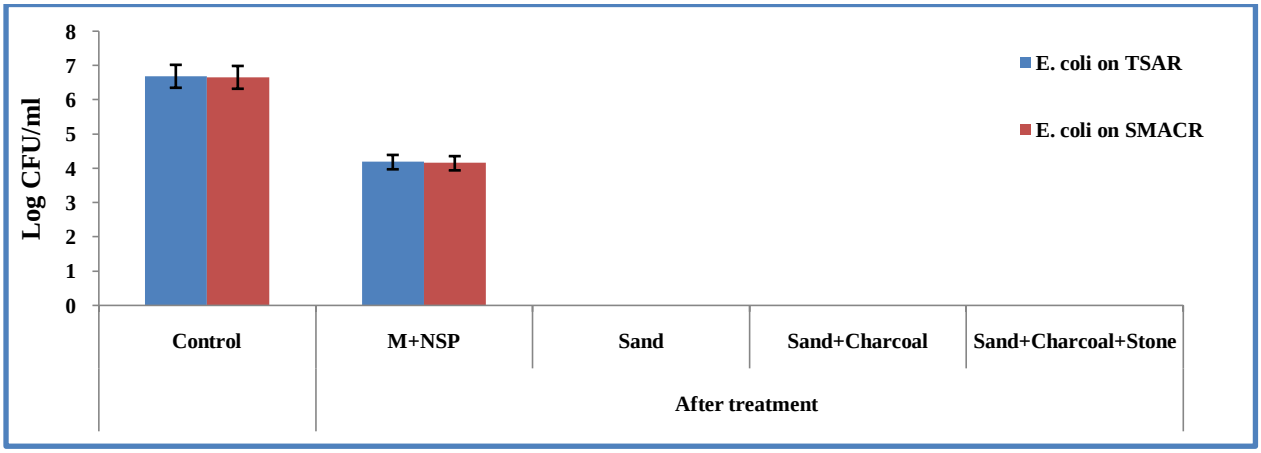


Figure 3.26: Effect of moringa seed powder, scallop powder and sand-charcoal-gravel filter on *E. coli* count (TSAR and SMACR) of rifampicin resistant bacteria (marker bacteria) of river water

3.4.2.1 Shelf-life study of spiked samples treated with moringa seed powder, scallop powder followed by bio-sand filtration

Ambient temperature (26-28°C) was maintained to preserve the control and treated water samples for 6 months and survivability of rifampicin resistant *E. coli* were analyzed. Results are represented through bar diagram in figure 3.27, 3.28 and 3.29 for pond, lake and river water samples respectively.

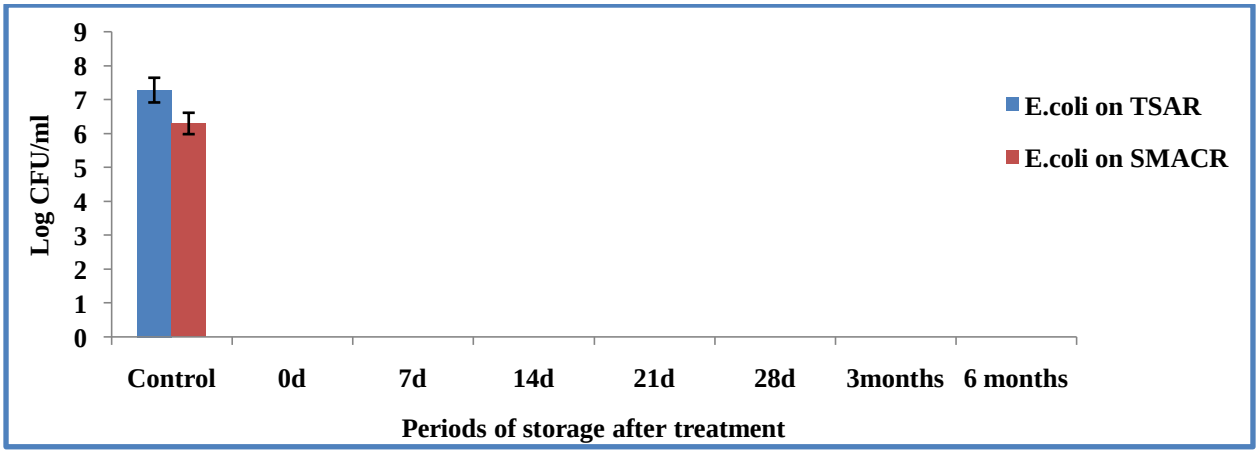


Figure 3.27: Bacterial (spiked) quality of 6 months stored treated pond water at ambient temperature

Irrespective of sources and types of sample water, presence of marker bacteria was being noticed before treatment.

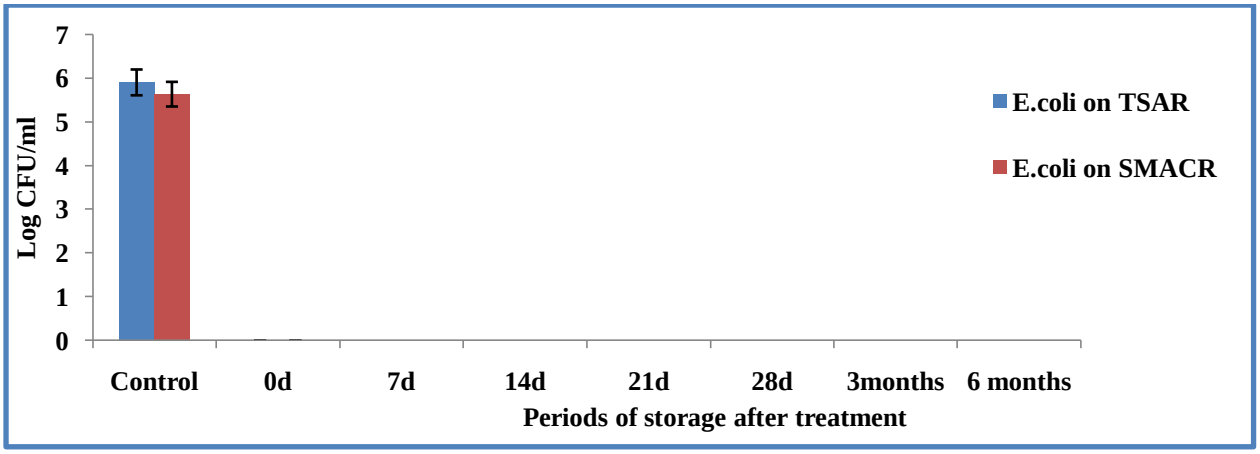


Figure 3.28: Bacterial (spiked) quality of 6 months stored treated Dhanmondi Lake water at ambient temperature

For all the sample types, results showed that, our moringa seed powder, scallop powder along with bio-sand filtration treatment were able to halt the rifampicin resistant *E. coli* resuscitation, thus no bacterial cell came into detection limit throughout the storage study.

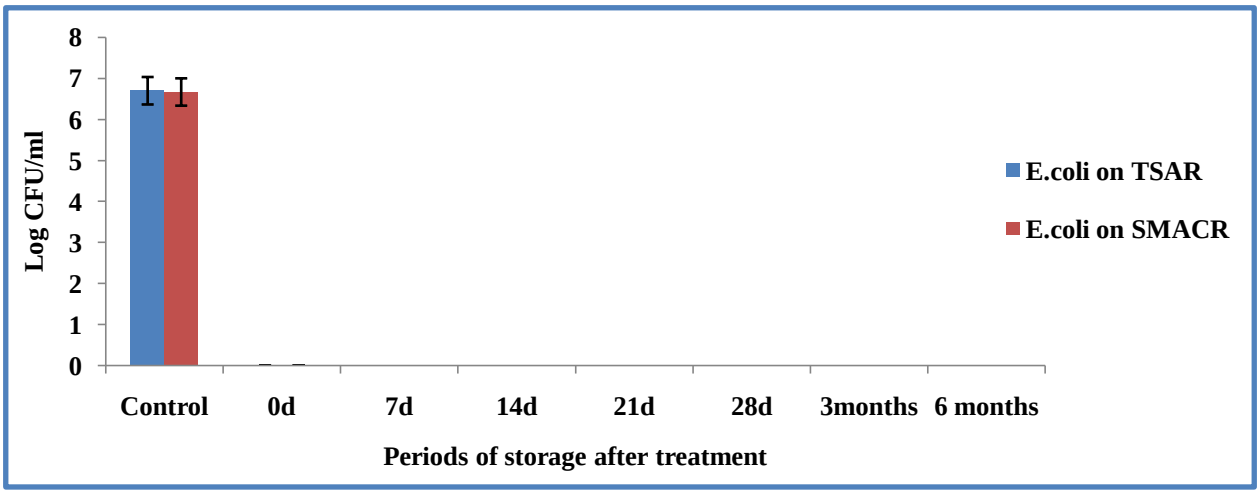


Figure 3.29: Bacterial (spiked) quality of 6 months stored treated river water at ambient temperature

3.4.3 Physico-chemical parameters of control and treated water samples (Treated with moringa seed powder, scallop powder followed by 3-step bio-sand filtration)

Seven environmental water samples were examined for physico-chemical parameters. The physico-chemical properties including color, odor, taste, pH, turbidity, TDS, BOD, COD, conductivity, major metal (Fe, Na, Ca, Mg and Zn) contents, heavy metals (As, Cd, Cr, Pb) contents were done according to the USEPA (2015) and BSTI (2001) standard methods. All the values were within the acceptable limit.

Table 3.3: Physico-chemical Parameters of treated and untreated samples along with USEPA and BSTI Standard (Treated with moringa seed powder, scallop powder followed by 3-step bio-sand filtration)

Physico-chemical Parameter	Mean value of control samples	Mean value of treated samples	EPA guidelines limits for drinking water	BDS standard
Color	Gray to yellowish (15-19 color unit)	12-14 (color unit)	15 (color unit)	5(Haxen unit)
Odor	Odorous	Odorless	3 threshold odor number	Unobjectionable
Taste	Fishy-sour smelly	Agreeable	Agreeable	Agreeable
pH	7.62-8.01	7.37-7.89	6.5-8.5	6.4-7.4
Salinity (ppt)	0.1	0.1	0.1	0.1
Turbidity	10.9-14.4 NTU	0.9-3.8 NTU	0.5-1.00 NTU	5 NTU
TDS	259-374mg/l	232mg/l	500mg/L	500mg/L
Conductivity µs/cm	750 -780	623-645	NS	NS
BOD	2.1-2.5mg/l	2.71-2.76mg/l	5mg/L	5mg/L
COD	2.214-3.195mg/l	2.27-2.31mg/l	40mg/L	40mg/L
Iron (mg/L)	0.00	0.00	0.3	0.30
Manganese (mg/L)	0.00	0.38	0.05	0.50
Lead (mg/L)	0.00036-0.00038	0.00046-0.00049	0.015	0.01
Zinc (mg/L)	0.00	0.08-0.10	5.00	3.00
Arsenic (mg/L)	0.00223-0.00228	0.00190-0.00197	0.01	0.05
Cadmium (mg/L)	0.00	0.00	0.005	0.003

*NS: Non selected

3.4.3.1 Comparison of turbidity value of raw water and MOSP treated water followed by filtration

Table 3.4: Comparative turbidity value of control and MOSP treated water followed by bio-sand filtration: Irrespective of water sources, higher turbidity ranging from 54.0 to 59.0 NTU was observed in raw water samples. Sari filtration alone can reduce the turbidity to 38.0–42.0 NTU or 30.0% and MOSP treatment further reduced the turbidity to 50% and MOSP treatment followed by cotton sari filtration was able to reduce it to approximately 70%. Goyal, 2015 has shown that on passing a water sample through a cotton cloth folded over three times (eight layers), the turbidity of that water sample was reduced by 48.23%. This finding is also similar to this study of turbidity reduction using cotton sari cloths. In contrast, bio-sand filtration alone was able to reduce the turbidity by up to 80% and moringa, scallop powder treatment followed by bio-sand filtration was able to reduce the turbidity by up to 99.9% and hence would be applicable for water filtration as the best filtration method compared to cotton sari filtration

Treatment Conditions	Turbidity value (NTU)*	US EPA Std turbidity value (NTU) for drinking water	BSTI Standard turbidity value (NTU) for drinking water
Control (non-treated)	54.0-59.0	0.5-1.00	5.0
Sari filtration (8 layer)	38.0-42.0		
Sari filtration followed by Moringa, scallop powder treatment	30.0-35.0		
Moringa, scallop powder treated water	26.0-30.0		
Moringa, scallop powdertreated followed by Sari filtration	20.0-25.0		
Bio-sand filtration only	10.9-14.4		
Moringa, scallop powder treatment followed by bio sand filtration	0.9 - 4.1		

*Average value range of raw water from pond, river and lake.

3.4.4 Detection of *uidA* gene by DNA extraction, PCR and Agarose gel electrophoresis (treated with moringa seed powder, scallop powder followed by 3-step bio-sand filtration):Boiled template DNA Extraction method (Medici *et al*., 2003) was followed in order to extract and detect *uidA* gene by PCR and agarose gel electrophoresis. Control samples gave specific bands (166bp), where treated samples were negative respective to *uidA* gene marker

(figure 3.30). *uid A* gene that code for β- glucuronidase, expression of which forms the basis for faecal coliform detection.

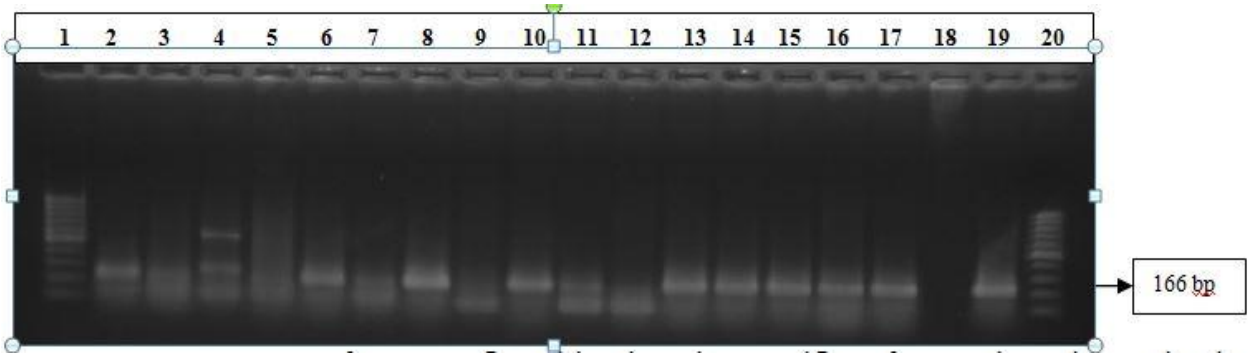


Figure 3.30: Representative *E. coli* was confirmed by detecting specific *uidA* gene using molecular method. Lanes: 1 and 20, represents 100-bp standard DNA ladder; Lane 2, 4, 6, 8, 10, 11, 13, 14, 15, 16, 17 represents non-treated samples; Lane 3, 5, 7, 9, 12 represents treated samples; Lane 18, negative control; & Lane 19 positive control

3.4.5 Cytotoxic effect of moringa seed powder and scallop powder on HeLa and BHK-21 cell

Cytotoxic effect of moringa powder and scallop powder was done on BHK-21 and HeLa cell using tissue culture methods at the cell culture laboratory of the Centre for Advanced Research in Sciences, University of Dhaka. Results are shown in figure 3.31 revealed that after moringa and scallop powder treatment, survival rate of BHK and HeLa cell was ~95%, which is the evidence of nontoxicity of both moringa and scallop powder.

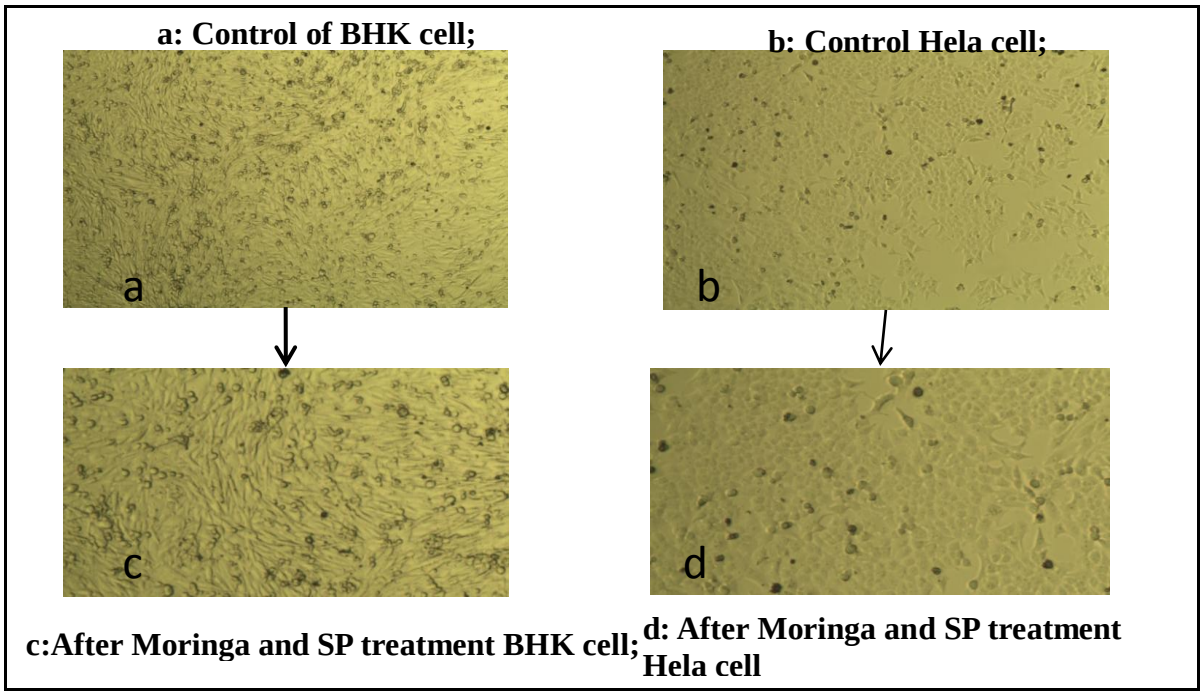


Figure 3.31: Cytotoxicity test of moringa seed powder and scallop powder on BHK-21 and HeLa cell

3.5 Pyrogen detection test using LAL test (gel clot method):

The endotoxins detection was done by LAL (Limulus Amebocyte Lysate) test, using a lysate of amebocytes isolated from the horseshoe crab (Limulus polyphemus) clotting reaction. This reaction involves coagulation cascade of sequentially activated proteases which formed clot with endotoxin. Both the non-treated and treated water sample was tested for bacterial endotoxins (figure 3.32). All the treated samples showed no gel formation after adding LAL reagent and incubation, which indicates the samples were free from bacterial endotoxins.

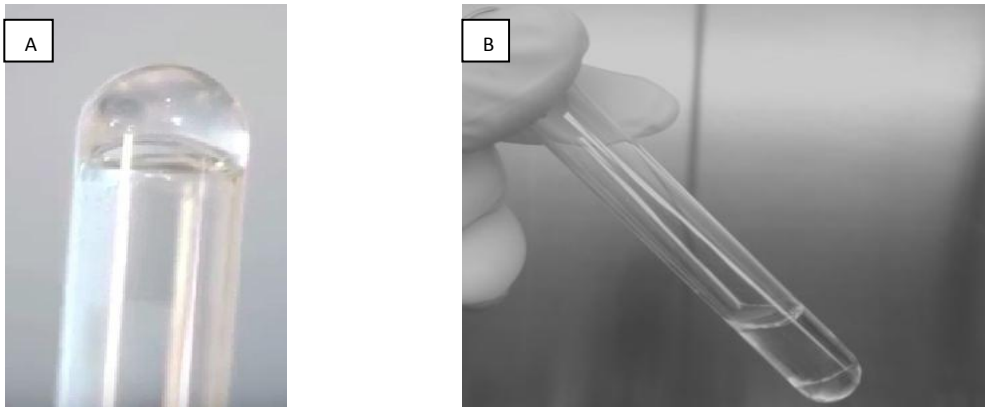


Figure 3.32: (A) represents the positive control (*E. coli* O111:B4) result showing the gel clot after adding the LAL reagent and incubation; (B) represents the negative result of test sample showing no gel formation after addition of LAL reagent and incubation

3.6 Comparison of scanning electron micrograph of a single eight layer of new and old cotton sari

A clean cotton cloth, typically called cotton sari in south asia was used for straining water samples, was examined under the scanning electron microscopyand the result is in the figure 3.33.The figure showed that an old sari cloth made up of cotton less pore size than the new sari, because threads of an old sari become soft and loose, reducing the pore size compared to a new cotton sari cloth which were visible in new sari but were not visible in the old or used sari.

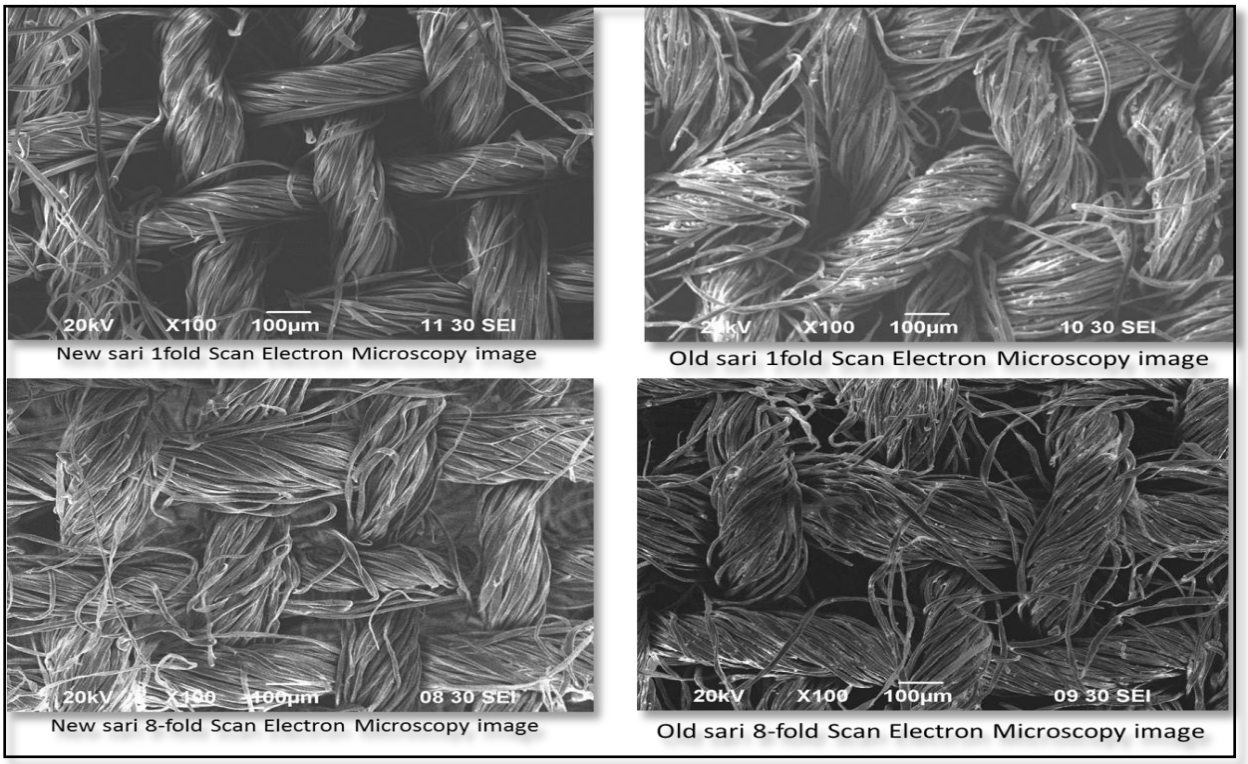


Figure 3.33: Comparison of scanning electron micrographs of a 4 layer and 8-layer of new and old cotton sari

3.7 Simple metals in inactivation of diarrhoeal pathogens of surface water

I.Use of Brass granules: Table 3.4 gives the results using small amounts of water (5 ml, 50 ml and 100 ml) in a test tube with 1 g of brass filings (granular). The water samples (pond water) were kept at rest or shaken for 30 minutes (50rpm) and then microbiological analysis were done. The result shows that, for 1 g brass granules in 5 ml water without shaking, total coliform and *E. coli* counts were inactivated after 30 minutes of treatment. However, with 50 ml and 100ml of water, inactivation was only possible with shaking for 30 minutes. The samples without shaking showed significant reduction in counts but cannot be considered safe for drinking from a microbiological point of view.

Table 3.5: Average result of three pond water after treating with brass filings

Microorganisms	Water (5 ml)		Water (50ml)			Water (100ml)		
	Average microbial population (Log CFU/ml)*							
	Control	Not shaken	Control	Not shaken	Shaken 30 min	Control	Not Shaken	Shaken 30 min
Total aerobic bacteria	3.08	2.03	3.14	1.96	1.76	3.38	2.43	1.49
Total coliform bacteria	2.76	**ND**	2.54	1.15	0.30	2.32	1.86	**ND**
Escherichia coli	2.57	**ND**	2.07	1.07	0.30	2.28	1.75	**ND**

*Average of three samples were analyzed in each water volume; ND= not detected; Detection limit <1 Log CFU/ml.

II. Use of brass, copper and zinc plates: Table 3.5 gives the comparative results of the three metallic plates with small amounts of water (50 ml and 100 ml), taken in plastic boxes. All the samples were shaken by hand, or using a shaker machine (50 rpm) later, continuously for 30 minutes after which the samples were subjected to microbiological analysis. In the comparative study of copper, brass and zinc in table 3.4, for 50 ml of water, which only created a thin layer over the 11cm x 7 cm plates and shaken for 30 minutes. All the three metals used (50 ml water) in this study were seen equally effective in reducing *E. coli* population to below detectable range. However, the brass was found reduced the coliform counts to non-detectable level, but larger water volume (100ml) was found not effective under similar experimental conditions. The brass and zinc used in 100 ml study was not found effective in reducing coliform bacteria, in contrast, copper showed higher effectivity in reducing *E. coli*.

Table 3.6 showed the results for a larger amount of water, 400 ml, in which the water was left undisturbed in the boxes with the respective metallic plates at the bottom for periods of 48 hours. Small samples of water were collected for microbiological analysis at 24 and 48 hours. Copper appears to be effective in inactivating *E. coli* successfully while brass and zinc could not, although the counts decreased for all, compared to the control water samples.

Table 3.6: Average result of three pond water after treating with copper, brass and zinc metallic sheets

Microorganisms	Water (50ml) shaken for 30 min				Water (100ml), shaken for 30 min			
	Average microbial population (Log CFU/ml)*							
	Control	Copper	Brass	Zinc	Control	Copper	Brass	Zinc
Total aerobic bacteria	3.32	2.82	2.61	2.97	3.01	2.40	2.21	2.33
Total coliform bacteria	2.78	0.60	**ND**	1.89	2.04	0.30	1.20	1.20
E. coli	2.42	**ND**	**ND**	**ND**	1.87	**ND**	0.70	0.60

*Average of three samples were analyzed in each water volume; ND= not detected; Detection limit <1 Log CFU/ml.

Table 3.7: Average result of three pond water after treating with copper, brass and zinc metallic sheets (up to 48 hours incubation)

Microorganisms	Water (400ml) Not shaken						
	Average microbial population (Log CFU/ml)*						
	24 hours incubation				48 Hours incubation		
	Control	Copper	Brass	Zinc	Copper	Brass	Zinc
Total aerobic bacteria	**3.33**	**2.71**	**2.85**	**2.88**	**2.65**	**2.83**	**2.82**
Total coliform bacteria	**3.06**	**2.45**	**2.56**	**2.71**	**2.38**	**2.54**	**2.68**
E. coli	**2.945**	**ND**	**2.475**	**2.525**	**ND**	**2.43**	**2.42**

*Average of three samples were analyzed in each water volume; ND= not detected; Detection limit <1 Log CFU/ml.

III. Effectiveness of inactivation of copper plates in the long term

Environmental water samples: After comparing the result of copper, brass and zinc metallic sheets, it seems that, copper was the best in killing the indicator bacteria, *E. coli*. So, the research work has been proceeding to disinfection of pond water using copper plates and storage of treated water samples.One container (sample-A) was left in a cool and dry place at room temperature with copper plate for 24 hours (microbiological study was done after 24 hours and then copper plate was removed), while another one was used for periodic microbiological study of 4 and 24 hours of treatment (sample-B), while copper plate was removed after 4 hours of treatment. Treated water was stored at room temperature (26-28 °C) without the copper plate for 28 days and periodic count was performed with 7 days intervals. In this experiment, all the resident bacteria along with coliform and *E. coli* were found to increase after storage at room temperature (figure 3.34, 3.35). Inoculation study also resembles with the environmental study (figure 3.36).Explanation of this result is that, copper injured the bacteria for some period and at that time bacteria were in VBNC (viable but non culturable) stage but during the storage, they recovered and came to culturable state.

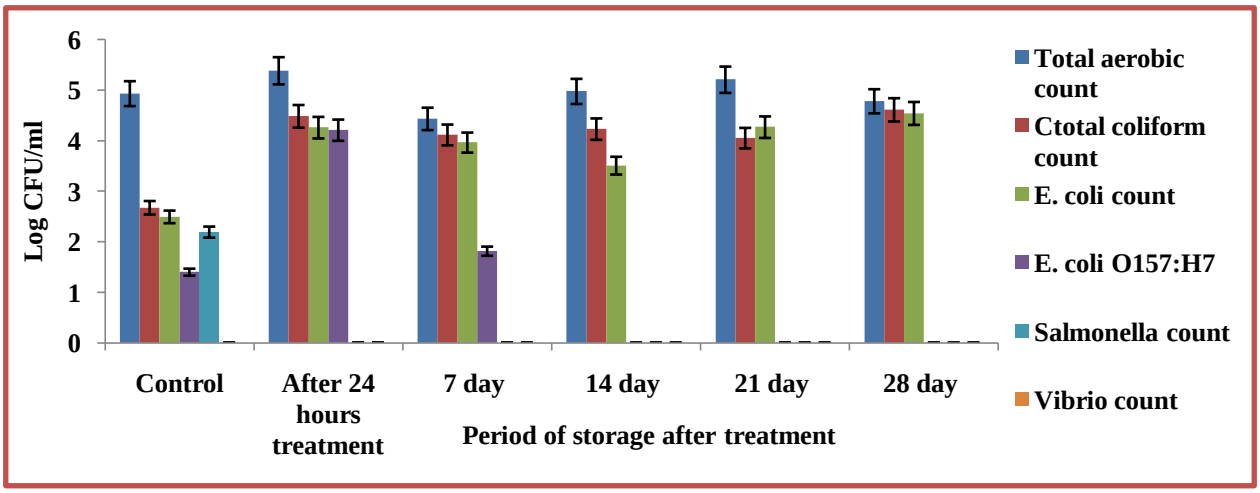


Figure 3.34: Effect of copper plate on total aerobic, total coliform, *E. coli*, *Salmonella* spp., *Vibrio* spp. count on pond water (sample-A)

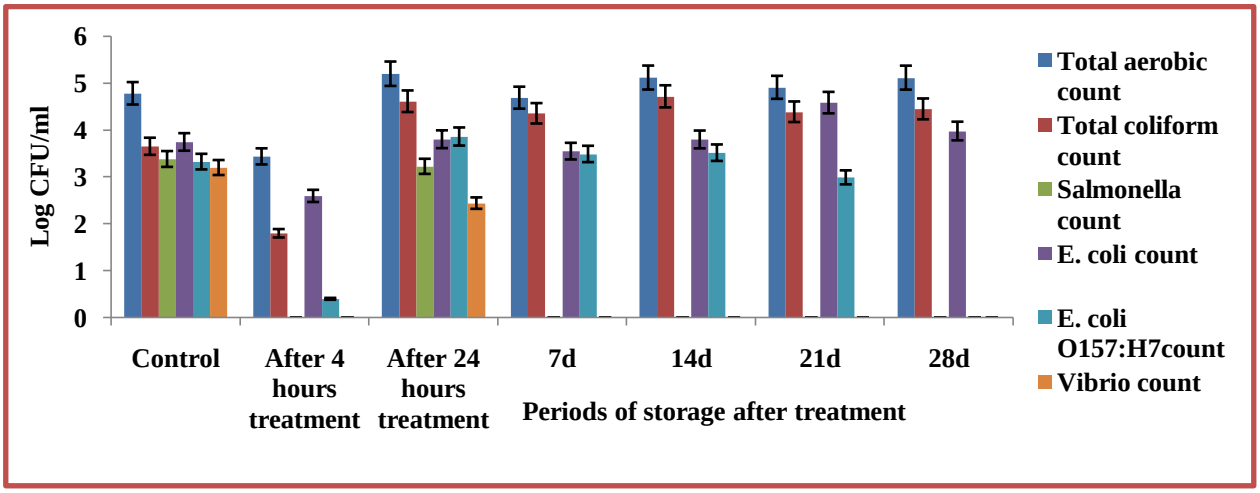


Figure 3.35: Effect of copper plate on total aerobic, total coliform, *E. coli*, *Salmonella* spp., *Vibrio* spp. count on pond water (sample-B)

- **Spiked Study:** Spiked/ inoculation study was done whether copper plate has the disinfection effect on rifampicin resistant *E. coli* (figure 3.36). Like environmental samples, marker bacteria also resuscitated on storage.

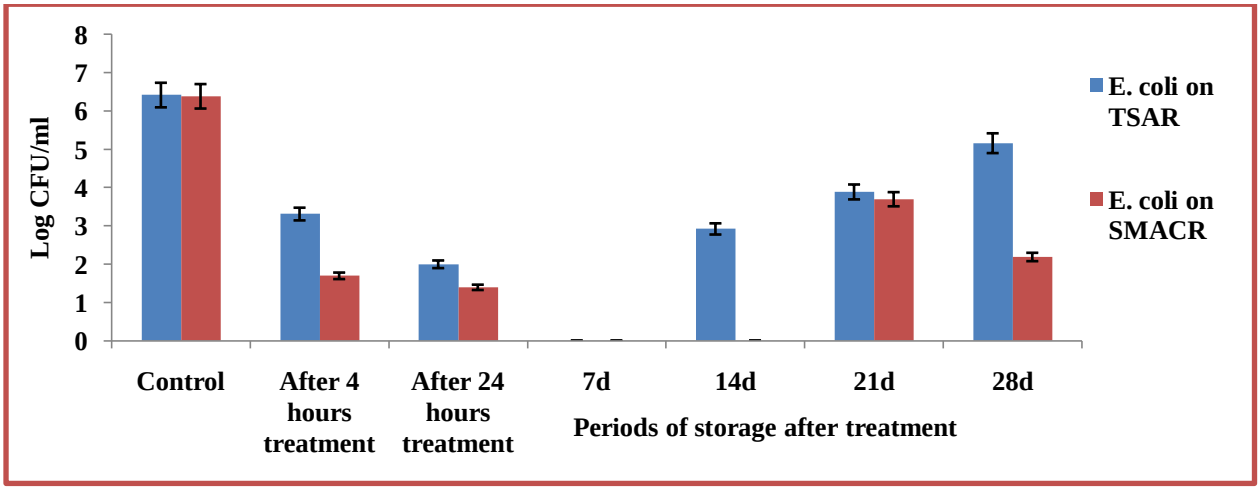


Figure 3.36: Effect of copper plates on rifampicin resistant *E. coli* count (TSAR and SMACR) of pond water

4.0 Discussion

Ponds, lakes and rivers are the main sources of surface water and surface water provide water resources for domestic, industrial and agricultural purposes (Ngwenya, 2006). Bangladesh is a riverine country and about 700 rivers are criss-crossed over the country. People have been using both ground water and surface water in their daily purposes, household works, agriculture, industrial sectors etc. Groundwater is the most important source of piped water in Bangladesh except for few hilly regions, but due to anthropogenic problem arsenic contamination and salinity concentration in underground water is increasing. On the other hand, excessive extraction of water leads to lowering the underground water level and arsenic problem is created. To cope with the situation, Bangladesh must rely on surface water from upland lakes, rivers and ponds which is mainly contaminated with enteropathogenic organisms. Other physico-chemical parameters are usually within the permissible limit for irrigation or household uses. Therefore, boiling water before drinking is effectively the better practice, because it kills all the waterborne pathogenic microorganisms. However, in the rural areas of Bangladesh, boiling practices are not routinely used, because of fuel and wood crisis. In Bangladesh, village women usually use cloth, frequently a flat, unfolded piece of an old sari, to filter home-prepared drinks. Hence, the research work includes filtration of water through sari, then treatment using different low-cost device materials and natural coagulant and antibacterial powder.

4.1 Effect of cotton sari filter on the quality of water

After collection of water samples, 8 layer/4 folded cotton sari, a material readily available to village women in Bangladesh, was used for pre-treatment filtration. Extensive experiments on cotton sari filtration were also done by colwell *et al.,* 2003 and the results of this study also resembles with that research group. In laboratory experiments using scanning electron microscopy, it was found that, an inexpensive cotton sari cloth, folded to four times provides a filter of about 20 µm mesh size, was small enough to remove all zooplankton, most phytoplankton, all *Vibrio cholerae* attached to the plankton and other particulates larger than 20 µm. Eight layers of cotton sari cloth were considered optimal for water filtration, since several research reports showed that 8-layer cotton sari consistently removed more than 99% of the bacteria, but 8 layers were approximately equally effective (Colwell *et al*., 2003). The scan electron micrograph image showing the comparison of a single and 8-layer of new and old sari presented in figure3.33 showed that an old sari cloth made up of cotton less pore size than the

new sari, because threads of an old sari become soft and loose, reducing the pore size compared to a new cotton sari cloth which were visible in new sari but were not visible in the old or used sari (Figure 3.33).

The physico-chemical parameters of the sari-filtered water remain unchanged except for turbidity. The turbidity was reduced to 38.0-42.0 NTU, above the permissible limit (figure 3.3). The bacterial count was reduced but the presence of *E. coli* and *Salmonella* spp. was evident in some samples, indicating that elimination of waterborne pathogens is not possible with just sari filtration. Using garment cloth such as linen, cotton and other cloth folded several times is a simple way for water purification. While it filters solid particles, microbes and pathogens larger than 20 microns, including 99% of the *V. cholerae*, it does not remove chemical contaminants or dissolved compounds which are less than 20 microns (Colwell *et al*., 2003).

4.2 Solar pasteurization device

Solar pasteurization device is basically a flat plate solar water heater, which is based on green house effect mechanism. In this device, heat and UV-light contribute to the disinfection of the water. So, the most dependable factor of this device is weather and the time of the year. On the sunny day, when sunlight intensity is high, the water will be heated faster. Otherwise, on a cloudy day, water sample might not reach to the same temperature as sunny day; still UV-light will destroy the pathogens even at lower temperature (Rabbani, 2002). As because the treatment process was under the disaster preparedness plan, so the shelf life study was extended upto six months.

The results of physico-chemical quality of raw and treated water have been presented in table 3.2. Physical and chemical quality parameters of surface water collected from different sources in this study revealed that the raw water is suitable for aquatic life, irrigation and domestic purposes without any form of treatment. Most of the parameters were found within the permissible limit of USEPA (2015) and BSTI (2001) standard except turbidity and microbial presence, thus not suitable for drinking purpose. The innovative solar pasteurization device along with four fold cotton sari filter fulfilled the requirements of drinking water criteria. All the parameters were within the USEPA and BDS standard limit.

Spiked study was done using environmental *E. coli* strain. *E. coli* was artificially inoculated with rifampicin antibiotic and use for the study (figure 3.10). The procedure was mentioned in the figure 2.5 of methodology.

In case of artificially inoculated *E. coli*, TSAR and SMACR count were recorded as5.6 and 5.4 log CFU/ml (pond), 4.6 and 4.4 log CFU/ml (lake), 6.8 and 6.7 log CFU/ml (river) respectively. The weather conditions of the treatment day were observed as clear, sunny, high humidity etc., and highest sunlight was recorded as1100 to 1200w/sqm.

The initial day temperature was recorded between 29-35 °C at 10.25-11.40 hour and temperature reached to maximum 64-74 °C at the treatment point of pond water at 14.25 to15.40 hour. As the temperature increased, the initial count of *E. coli* population on TSAR and SMACR declined to 0.66 and 0.63 log CFU/ml in case of pond water sample after 1.0 hour of exposure and then reached to lower than detection limit (Figure 3.11). Otherwise, in case of lake and river water samples, *E. coli* population on TSAR and SMACR reached to below the detection limit after 1.0 hour of exposure and thereafter (Figure 3.12, 3.13)

Room temperature was maintained for six months in storing the treated water samples and rifampicin resistant *E. coli* count were below the detection limit throughout the study (Figure 3.14, 3.15, 3.16).

Several other studies have been conducted using different types of low-cost solar pasteurization device. Onyango *et al.*, 2009 used car radiator thermostat valve, copper pipes, galvanized pipes etc., which are cost intensive than our materials (Hay, bamboo tray, polythene sheets). Storage studies of treated water also strengthen our technologies appeal. Rojko *et al.*, 2003 also studied on solar disinfection method on the artificially inoculated *E. coli* in water samples. Otherwise, spiked study of our work including six months storage of treated water strengthens the effectiveness of the solar pasteurization device.

Two of the most significant factors are the intensity of solar radiation and duration of exposure to have a safe water quality. Since insulation at the bottom and the air gap between the top transparent sheets are important, these were maintained with due caution. Solar radiation is the general source of heat, and necessary to achieve an adequate water treatment, but if the device lacks in insulation, considerable amount of heat loss may occur and the temperature will not increase as desired. Greater thickness of insulation at the bottom and double air gap at the top can hold the solar energy longer and thus water temperature will be higher.

Major difficulties, during performing the solar radiation test were, the maintenance of the identical initial temperature for all tests, since the outdoor temperature was not as constant as the indoor temperature. The sample preparation time before starting the tests varied slightly each time that affected the initial water temperature. Despite the time variation in preparation, other impacts include the initial device temperature also varied due to the solar radiation. These issues could explain the small differences in the results, but the major result of importance is that if clear sunshine is available for about two hours, it is enough to destroy all enteropathogenic bacterial population in water making it safe to drink.

The microbiological studies showed that the low cost solar pasteurization device can make pond water safe to drink. Solar radiation has the effect of preheating the surface water in the pond, lakes and rivers; this might have reduced pathogens already before treatment. This could be the reason for the relatively lower concentration of pathogens in the control samples. On a typical clear sunny day in Bangladesh (during February to July), when the solar radiation is the strong, the simple device tested could disinfect (pasteurize) water three times a day producing about 15 liters per day. In the winter (December to February) when the solar radiation is less strong, then about 10 liters may be obtained per day with two treatment sessions.

The cost of the solar water pasteurizer would be around 400 BDT, equivalent to US$ 5.0, which is very low cost. In addition, the device is made of black colored bamboo tray, straw, polyethylene sheets, polyethylene bags, which are readily available in the rural areas. If proper training is provided to rural people before floods and organize these required materials, they can be able to use this device on rafts or at emergency shelters during floods.

4.3 Moringa seed powder, scallop powder and bio-sand (sand-charcoal-gravel) filter

The *Moringa oleifera* seeds remove turbidity through coagulation as well as antimicrobial activity is well documented (Taraba *et al*.,1990). Heated scallop shell powder has been shown to deactivate and remove many foodborne pathogens like *E. coli*, *Salmonella* spp., *Listeria* spp. and *Staphylococcus aureus* (WHO, 2013).

Bio-sand filters have been shown to remove 90-99% of pathogens found in water. The filter has been tested by various government organizations, research, and health institutions, as well as by non-governmental agencies in both laboratory and field settings. Overall, many studies have shown that the bio-sand filter removes, more than 97% of *E. coli* - an indicator of fecal

contamination (Duke *et al*., 2006), 99% of protozoa and helminths (Palmateer *et al*., 1999); 80-90% of viruses (Stauber *et al.*, 2006); 50-90% of organic and inorganic toxicants (Palmateer *et al*., 1999); 90-95% of iron (Ngai *et al*., 2007) and most suspended sediments. The bio-sand filter may also remove some heavy metals (Muhammad *et al*., 1997). A modified bio-sand filtration was found effective in removing both pathogens and 85-90% of arsenic from source water (Ngai *et al*., 2007). Preliminary health impact studies estimate a 30-40% reduction in diarrhea among all age groups, including children under the age of five, an especially vulnerable population (Liang *et al*., 2007).

The bio-sand filter is a modified form of the traditional slow sand filter in such a way that the filters can be built on a smaller scale and can be operated intermittently. These modifications make the bio-sand filter suitable for household or small group use. The bio-sand filter can be produced locally anywhere in the world using materials that are readily available.

Bacteriological analysis results showed that the pond water was grossly contaminated than lake and river water as because pond water is stagnant and there is no way to flow away the pollutants. The total coliform count and *E. coli,* indicative of faecal pollution, recorded as for pond 3.9 and 3.1 log CFU/ml respectively. However, lower level of coliform and *E. coli* count were observed in lake (2.95 and 2.94 log CFU/ml respectively) and river (3.3 and 3.0 log CFU/ml respectively) water.

On an average, irrespective of sample source, substantial number of resident bacteria (for pond 5.8 log CFU/ml, for lake 5.0 log CFU/ml and for river 4.1 log CFU/ml) was recorded in raw water (figure 3.18, 3.19, 3.20), but, presence of *Vibrio* spp. and *Salmonella* spp. were not found in the water samples analyzed. After treatment, irrespective of sources and type of water, none of these bacteria was detected in MOSP treated followed by bio-sand filtered water. Non-significant differences in most of the physico-chemical quality parameters were observed except for pH. When moringa and scallop powder was added to the water the resulting pH was recorded as 9.6-9.8. This water while passes through sand, charcoal and gravel comes in contact with organic matters and presence of charcoal might absorb some color and stone might add some minerals to the water and consequently reduced to neutral pH.

Turbidity is the measure of relative clarity of a liquid and the measurement of turbidity is a key test of water quality. In drinking water, the higher turbidity level may develop gastrointestinal disease (Mann *et al.*, 2007). Moreover, high turbidity in water is a possible source of microbial

contamination and it also reduces the efficiency of the water purification systems (Edition, 2011) The suspended solids interfere with water disinfection because the particles act as a shield for the virus and bacteria. Similarly, suspended solids can protect bacteria from ultraviolet (UV) sterilization of water. Irrespective of water sources, higher turbidity ranging from 54.0-59.0 NTU was observed in raw water samples. Sari filtration alone can reduced the turbidity to 38.0-42.0 NTU or 30.0% and MOSP treatment further reduced the turbidity to 50% and MOSP treatment followed by cotton sari filtration was able to reduce turbidity approximately 70%. Maneet G. (2015), have shown that on passing a water sample through a cotton cloth folded over three or four times (8-layers), the turbidity of that water sample was reduced by 48.23%. This finding is also similar to the result of turbidity reduction using cotton sari cloths. In contrast, bio-sand filtration alone was able to reduce the turbidity up to 80% and MOSP treatment followed by bio-sand filtration was able to reduce the turbidity up to 99.9% and hence would be applicable for water filtration as best filtration method compared to cotton sari filtration. According to the World Health Organization, the turbidity of drinking water should ideally be less than 1.0 NTU. Water with turbidity more than 1.0 NTU may be safe for drinking, but the visible cloudiness has a negative impact on consumer acceptability (Table 3.4).

Toxicity test data reveal that the natural coagulant and natural antimicrobials used in this study did not have toxic effect on BHK-21 or HeLa cells (Figure 3.31) which indicates the materials safety and doesn't pose any health effect. Several studies have found moringa and scallop powder to be non-toxic (Amagloh *et al.,* 2009; Liu *et al.,* 2006). However, chlorine frequently used, has carcinogenic impact on human health, thus use of non-hazardous, non-toxic, antimicrobial and coagulant could be introduced instead of chemical antimicrobials for water disinfection.

Figure 2.4 of methodology section represents household scale filtration unit and the results of the analysis of the raw water after treating with varying amount of MOSP. The MOSP dose of 0.1% was found to be optimal for the reduction of turbidity. It was also observed that most of the larger flocs settled faster at the dosage of 0.1%. Further reduction of turbidity 10 ± 0.6 NTU was recorded for bio-sand filtered samples. The treatment with MOSP alone gave a percentage turbidity reduction of 95.55% while the MOSP and filtration method gave 97.75% reduction in turbidity values.

While MOSP alone can be used to clarify water, a combination of MOSP and household sand filter was found to be more effective in the removal of turbidity and reduction of total coliforms

and *E. coli*. The reduction is confirmed statistically at 5% level of significance. Similarly, bacteriological data showed that the MOSP+F treated sample revealed a high percentage reduction of total coliforms 99.94% and *E. coli* of 99.97%. This is an indication that the combined (MOSP+F) treatment method had the advantage of reducing microbial load better than the single treatment. The combined method gave the lowest coliform count per 100 ml of the samples as compared to the MOSP treatment method. Irrespective of sources and types of water, when MOSP + bio-sand filtered water was stored at room temperature for 6 months none of the bacteria was detected throughout the study (Figure 3.21, 3.22, 3.23). This finding suggested that the methodology used in this study could be useful for household level.

Spiked/inoculation/challenge study results also resemble with the environmental study. After inoculation, *E. coli* on TSAR and SMACR was recorded as 7.3 and 6.3 log CFU/ml (pond sample, figure 3.24), 5.8 and 5.5 log CFU/ml (lake water sample, figure 3.25), 6.7 and 6.6 log CFU/ml (river water sample, figure 3.26). Irrespective of sources and types of water, no *E. coli* was detected in MOSP treatment followed by bio-sand filtered water.

Surface water treated with combined moringa seed powder, and scallop powder (MOSP) followed by natural bio-sand filtration was found producing drinking quality water. The bacteriological, physico-chemical quality parameters (figure 3.2) and storage study of this water showed non-significant differences in quality parameters and 6 months stored water at room temperature was achieved without compromising the quality, indicating the usefulness of this technology in drinking water scarcity areas of the world, because the ingredients used are readily available, inexpensive, user friendly and natural. Therefore, despite many similar water purification systems available commercially and several studies have been found related to this study (Mumuni *et al.,* 2013; Yongabi *et al.,* 2011), but this new methods would be the simplest, inexpensive and eco-friendly. The people can easily get the drinking water if this technology and training provided.

Many research papers have been published on surface water purification using moringa seed powder and sand filtration (Mumuni *et al.,* 2013; Yongabi *et al.,* 2011). The superiority of our work over those work are- combination of every ingredients i.e. moringa seed powder was used for coagulation, otherwise, turbidity was not removed by next ingredient scallop powder. Scallop powder is a strong antibacterial agent but at the same time increase the pH (9.5 to 10.5) of water sample. Sand decrease the alkaline pH to neutral pH range, charcoal has a magic property of odor removing as well as decoloring. Gravel gave the mineral and natural taste after

treatment. So, every ingredient has a specific reason to add up with this combined purification system. Moreover, six-month storage study made this purification system to be unique and more acceptable to the water scarcity areas.

The occurrence of coliform and *E. coli* in water indicates fecal contamination and is considered as the potential risk of diarrheal disease. Detection of these pathogens in water using conventional culture and biochemical assays is highly time consuming (18-96 hours), arduous and not reliable. Another significant constraint of *E. coli* is that the number of them can be altered in sample within less time period. As a result, detection by exploiting ß-glucuronidase activity of *E. coli* may give false positive or false negative results. Moreover, viable but non-culturable cells also remain uncountable in conventional culture method (Khan *et al.*, 2007). To crack the hindrance in detecting pathogens in water, molecular techniques come up as blessings. Use of polymerase chain reaction and agarose gel electrophoresis has turned out to be the most effective tools for detection. Applying PCR, a diminutive amount of specific DNA can be identified. To detect *E. coli*, several genes are targeted for PCR amplification such as, lacZ and lamb, *uidA*, 16s rRNA, gadAB and cyD. Among these genes, the housekeeping gene *uidA* is mostly used. This gene encodes for ß-glucuronidase enzyme which is a potential characteristic of *E. coli*. While PCR is conducted to identify *E. coli*, specific primers for *uidA* gene are used (Heijnen *et al.* 2006; Khan *et al.* 2007). Control samples gave specific bands in respect to 166bp marker, while treated samples were negative respective to *uidA* gene marker (Figure 3.17 for solar pasteurization device and figure 3.30 for moringa, scallop powder followed by bio-sand treatment). So, the molecular technique also confirms the cultural technique.

During cell division and lysis of cells, endotoxin released. Endotoxin is a part of lipopolysaccharide complex, that forms the outer envelope of Gram negative bacteria (Henderson,1996). Endotoxin is an essential part of quality assurance and quality control involves the testing end product of clinical and raw materials (Rosimar *et al*., 2004). Gram negative bacterial endotoxin infections can often cause a pyrogenic response. Limulus Amoebocyte Lysate (LAL) test is most popular technique of pyrogen detection test.

Only the positive control gave the gel clot positive result (*E. coli* O111:B4, Control Standard Endotoxin), while the other water sample gave the negative result showing no gel formation after addition of LAL reagent and incubation at 37 °C for 1 hour on heat block machine (figure 3.32). Dilution has been done to each sample, which is the easiest way to overcome the product

inhibition situation. As test samples may alter the optimal condition (specific pH, salt and divalent cation requirement) to lysate which may render insensitive to endotoxin.

4.4 Effectiveness of copper, brass and zinc metals on the quality of surface water

Technology for safe drinking water for rural areas of the low resource countries is still a big challenge for the humankind. The present study was designed to develop a simple technology, employing the bactericidal effect of metals that can be implemented by rural households. Copper and silver ions together have been known to have a bactericidal effect since ancient times, although the mechanisms are only surfacing in the recent times. Copper-silver ionization was developed in both Europe and the United States in the 1950's. Copper vessels have been reported to be effective in destroying the bacterial population especially *E. coli* (Sharan *et al*., 2010; Warnes *et al*., 2011; Dhanalakshmi *et al*., 2013). There has been a renewed interest in the use of copper and silver nanoparticles embedded in various base materials and have been claimed to disinfect water (Jain *et al*; 2005; Lin *et al*., 2013;Morones *et al*., 2005; University of Virginia,2014).Getting nanoparticles may be difficult in the rural areas of a low resource country. Therefore, instead of going for metallic nanoparticles, the department of Biomedical Physics and Technology tried using direct metals or their granules and the present work carried out the necessary investigation for this idea.

The above study suggests that coliform and *E. coli* were possibly injured and therefore inactivated temporarily due to the effect of copper, but resuscitation was observed after storage. This suggests that copper does not destroy bacteria, rather it inactivates them. However, this study was not performed for brass and zinc and therefore, nothing may be said about the effect of these metals at this stage. Several studies have suggested that, after incubation of water with copper, concentration of copper in water was increased over the permissible limit of World Health Organization (2.0 mg/l). Higher concentration of copper has been reported to cause diarrhoeal diseases, nausea and other symptoms (Dhanalakshmi *et al*., 2013; Pizarro *et al.*, 1999).So; further studies will be needed to find out whether these simple metals may be used for treatment of water for drinking.

4.5 Final words

It does not need any saying to emphasize the importance of safe drinking water. Many years back people used to drink water from rivers, ponds, wells, lakes etc, which was associated with

the risk of diarrhoeal diseases. Then, tubewells came as a Godsend, soon turned out to be a devil by bringing out arsenic poison from the ground below because of excessive use of ground water. Now, we need to go back to surface water reservoirs, which we were used to once. In the rural areas of third world countries like Bangladesh, there are no industries. The surface water of those areas is mostly free from industrial wastes, however, pesticides applied in agriculture, can be a problem. But this can be reduced by campaigning and creating the awareness about spread of such pesticides, so that water bodies are kept free of the pollutants. We can reserve the upland reservoirs in every village with raised edges, especially for drinking water. Then, by boiling this water, or using some innovative low-cost techniques, the rural people can themselves solve their problem of safe drinking water.

Sometimes, people get engrossed in seeking solutions through complex ways when simple solutions exist but are overlooked. Millions of dollars have been spent for finding a solution to arsenic in ground water, but no simple solution has come by so far. We, the educated group, have immense responsibility towards the common people in our respective countries, particularly to the majority of people living in the villages. Because the poor people sacrificed to educate us in modern science and technology. It is our responsibility to deliver the benefits of modern science and technology to their doors. The research and efforts of this work was based on this philosophy. The technologies that have been presented in this study can all be done by rural people at home, using materials available around their house or in the local markets. Otherwise, community based ones could be possible for economical savings. If a family or community store the items needed to make water safe for drinking in a safe place, they can use these during and after the disaster. This is how; these can be a part of the disaster preparedness plan for a family or community with prior training.

5.0 Concluding Remarks

People living in the rural areas of most low resource countries are deprived of the basic necessities of life because of illiteracy, communication infrastructure and indifference of the richer and educated section of the people of these very countries. The present study has been carried out to evaluate and assess some simple and inexpensive surface water treatment systems intended to provide safe drinking water in rural areas of low resource countries. The systems chosen for this study can be easily used and maintained by the rural people in these countries.

- First technique is a low-cost solar pasteurization device that can destroy enteropathogenic bacteria in surface water to make it safe to drink, which was verified through this study. Microbiological and physico-chemical properties of the treated water were also within the limits of USEPA and BSTI (Bangladesh) standards. Molecular study and inoculation study also confirmed the results of the cultural study. During storage of treated water for 6 months, microbiological quality parameters were below the detection limit and physico-chemical parameters were also within the USEPA and BSTI (Bangladesh) standard limits.

- The second technique includes a natural coagulant (moringa seed powder) and a natural antibacterial agent (scallop powder) to treat water followed by natural filtration using sand, charcoal and gravel. This also removed enteropathogenic bacteria successfully from the treated water. The shelf life study of this water demonstrated that this water can be stored at room temperature up to 6 months without compromising the microbiological and physico-chemical quality, indicating the usefulness of this technology in the areas of the world where drinking water is scarce, because the ingredients used are readily available, inexpensive, user friendly and natural.

- The above mentioned two simple and easy treatment methods are particularly helpful for flood prone areas of Bangladesh, where there is a scary of drinking water during flood. The people can easily get the drinking water if training on these technologies is provided.

- Toxicity test data revealed that the natural coagulant and natural antimicrobials used in this study did not have toxic effect on BHK-21 or HeLa cells, which indicates the materials doesn't pose any health hazard.

- Pyrogen detection test using gel clot LAL test revealed that none of the sample showed presence of pyrogenic substance (gel formation) after addition of LAL reagent and incubation.

- The scanning electron micrograph image showing the comparison of new and old cotton sari. The image showed that an old cotton sari cloth made up of cotton had less pore size than the new cotton sari, because threads of an old cotton sari become soft and loose, reducing the pore size Eight- layer cotton sari cloth was used for pre-treatment filtration technique.

- The third technique evaluated for disinfecting surface water used granules or plates of simple inexpensive metals (copper, zinc and their alloy brass). The study revealed that, brass showed bactericidal effect with very small amount of water, with the whole volume almost touching its surface while it failed with larger amounts of water. The metal copper showed better performance than other metals in killing resident microorganisms throughout this study in large volumes of water. The shelf life study of the treated water using copper revealed that, the resident microorganisms were possibly injured due to the effect of copper, and showed a great reduction in counts immediately after treatment, but appeared to resuscitate during storage over days. Thus, the use of copper, brass or zinc metallic sheets was not able to inactivate the bacterial population effectively and thus, this study was not extended further.

6.0 References

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Appendix - I

Media Composition

The composition of the media used in the present study has been given below. Unless otherwise mentioned all media were autoclaved at 121 °C for 15 min.

1. Pre-enrichment (Nutrient broth 0.5 Strength):

Ingredients	Amount (g/L)
Peptone	2.5
NaCl	2.5
Beef extracts	0.5
Yeast extracts	1 .0
Distilled water	1 Liter
pH	7.1

2. Enrichment Media:

Ingredients	Amount (g/L)
Peptone	3.0
NaCl	l.0
K2HPO4	2.0
KH2PO4	l.0
Bile salt	0.015
Distilled water	1 Liter
pH	7.0

3. Tryptic Soy Agar:

Ingredients	Amount (g/L)
Peptone	5.0
Beef extracts	3.0
NaCl	5.0
Agar	15
Distilled water	1 Liter
pH	7.0

4. Chromocult Agar:

Ingredients	Amount (g/L)
Peptones	3.0
sodium chloride	5.0
sodium dihydrogen phosphate	2.2
disodium hydrogen phosphate	2.7
sodium pyruvate	1.0
tryptophan	1.0
Agar	10.0
Sorbitol	1.0
Tergitol®	7 0.15
chromogenic mixture	0.4
Distilled water	1 Liter
pH	6.8 ± 0.2

5. Sorbitol MacConkey Agar:

Ingredients	Amount (g/L)
Peptone	20.0
Sorbitol	10.0
NaCl	5.0
Bile salt no.3	1.5
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
Distilled water	1 Liter
pH	7.1±0.2

6. Bismuth Sulphite Agar (BSA):

Ingredients	Amount (g/L)
Peptone	5.0
Lab Lemco powder	5.0
Glucose	5.0
Disodium phosphate	4.0
Ferrous sulphate	0.3
Bismuth sulphite indicator	8.0
Brilliant green	0.016
Agar	12.7
Distilled water	1 Liter
pH	7.6±0.2

7. Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS agar):

Ingredients	Amount (g/L)
Sucrose	20.0
Dipeptone	10.0
Sodium Citrate	10.0
Sodium Thiosulfate	10.0
Sodium Chloride	10.0
Yeast Extract	5.0
Oxbile (Oxgall)	5.0
Sodium Cholate	3.0
Ferric Citrate	1.0
Bromothymol Blue	0.04
Thymol Blue	0.04
Agar	15.0
Distilled water	1 Liter
pH	8.6±0.2

8. Kligler Iron Agar (KIA):

Ingredients	Amount (g/L)
Beef extract	3.0
Yeast extracts	3.0
Peptone	15.0
Protease peptone	5.0
Lactose	l0.0
Dextrose	1.0
Ferrous sulfate	0.2
Protease peptone	5.0
Sodium chloride	5.0
Sodium thiosulfate	0.3
Phenol Red	0.024
BactoAgar	15.0
Distilled water	1 Liter
pH	7.4 ±0.2

9. Simmons' Citrate Agar:

Ingredients	Amount (g/L)
NaCl	5.0
MgSO4	0.2
NH4PO4	1.0
K2HPO4	l.0
Sodium citrate	l.0
Agar	20.0
Distilled water	1 Liter
pH	6.8±0.2

10. Motility Indole Urea (MIU):

Ingredients	Amount (g/L)
Peptone	30.0
KH2PO4	2.0
Sodium chloride	5.0
Phenol Red	0.005
Urea	20.0
Bacto agar	4.0
Distilled water	1 Liter
pH	7.3± 0.2

11. Luria Bertani Broth:

Ingredients	Amount (g/L)
Bacto tryptone	10.0
NaCl	10.0
Bacto yeast extracts	5.0
Distilled water	1 Liter
pH	7.4

12. T_1N_1 Soft Agar:

Ingredients	Amount (g/L)
Trypticase	10.0
NaCl	10.0
Agar	2.0
Distilled water	1 Liter
pH	7.5

Appendix - II

Buffers and Reagents Used

Preparation of the stock solutions, used in this work are given below; (all the working solutions used in this work were prepared from the stock solutions).

Normal Saline:

Normal Saline was prepared by dissolving 0.85 g NaCl in 100 ml of distilled water and sterilized by autoclaving, pH was adjusted to 7.8.

1M Tris-HCl:

121.1 g Tris-base was dissolved in 800 ml of distilled water. The pH was adjusted to the desired value by adding concentrated HC1 and the final volume was made up to 1 L with distilled water. The solution was sterilized by autoclaving and stored at room temperature (RT).

3 M NaCl:

175.3g of NaCl was dissolved in distilled water to a final volume of 1L. The solution was autoclaved and stored at room temperature (RT).

10 M NaOH:

40 g of NaOH pellet was dissolved in distilled water to final volume of 100 ml. The solution was stored in an airtight bottle at RT.

0.5M EDTA:

186.1 g of $Na_2EDTA.2H_2O$ (disodium ethylene diamine tetra-acetic acid)and 20 g of NaOH pellets were added to 800 ml distilled water and dissolved by stirring on a magnetic stirrer. The pH was adjusted to 8.0 with a few drops of 10M NaOH and final volume was made up to 1 L with distilled water. The solution was sterilized by autoclaving and stored at RT.

3M Sodium Acetate:

40.81g Na_2 $(CH_3COOH).H_2O$ was dissolved in 80 ml of distilled water. The pH was adjusted to 5.2 with glacial acetic acid. The final volume was adjusted to 100 mL with distilled water and the solution was sterilized by autoclaving. It was stored at 4 °C.

10% SDS:

10g of SDS (sodium dodecyl sulfate) (sigma) was added to 80ml of distilled water and dissolved by stirring on a magnetic stirrer slowly to avoid foaming. The final volume was adjusted to 100 mL with distilled water and stored at RT.

Phosphate buffer saline (PBS):

PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 800 mL of distilled water. pH was adjusted to 7.4 with HCl. The final volume was adjusted to 1L by distilled water. The solution was sterilized by autoclaving for 20 minutes and stored at 4°C.

TE Buffer:

10 mM Tris-Cl (pH 8.0), 1 mM EDTA was prepared by diluting concentrated stocks of 1M Tris-Cl (pH 8.0) and 0.5 M EDTA. The buffer was stored at 4 °C.

TAE- Buffer (50X and 1×):

242 g of Tris base, 57.1 mL of glacial acetic acid, 100 mL of 0.5M EDTA (pH 8.0) were taken and distilled water was added to the mixture to make 1 litre. 1× concentrated TAE buffer was made by adding 10mL 50X TAE buffer with 490 mL distilled water and stored at RT.

Ethidium Bromide Solution (staining solution):

Ten (10) µL of Ethidium bromide was dissolved in 100 mL TAE buffer to make a final concentration of 20 mg/mL and stored at 4 °C in the dark.

Gel Loading Buffer:

6% concentrated loading buffer consisted of 0.25% bromophenol blue, 0.25% xylene cyanol FF, 40% (w/v) sucrose in distilled water. It was stored at 4 °C.

0.5 McFarland Standard:

A 0.5 McFarland turbidity standard was prepared by adding 0.5 mL of 1.175% (w/v) barium chloride dehydrate ($BaCl_2.2H_2O$) solution to 99.5 mL of 1% sulfuric acid.

Appendix – III

Primers and Reaction mixture of PCR

PCR Primers:

uidA* gene for detection of *E. coli:

Forward: 5'-TAT GGA ATT TCG CCG ATT TT-3'

Reverse: 5'- TGT TTG CCT CCC TGC TGC GG-3'

Table A1: Reaction mixture for amplification of *uidA* gene of *E. coli:*

Components	Volume (µL)
Sterile deionized water	7.5
6x PCR buffer with 20 mM $MgCl_2$	1
10 mM dNTP mixture (10 mM each of dATP, dCTP, dGTP, dTTP)	0.2
Primer forward (10 mM each)	0.625
Primer reverse (10 mM each)	0.625
Taq DNA polymerase (5 U/µL)	0.05
Template DNA	2.5
Total	12.5

Appendix – IV

Apparatus used

Apparatus	Model/ Company
Autoclave	Hirayama model HL-42, AE, Japan
Centrifuge machine	Hitachi (CT10), Japan
Class-2 Al biological safety cabinet	SafeFAST Top, Italy
Duran bottle	Scott, Germany.
Electric balance	GR-120, A&D co, Ltd, Japan
Eppendorf tubes (1.5ml)	Abdos, India
Freezer (-20 °C)	Walton (FC-2T5), Bangladesh
Fridge (4 °C)	Walton, Bangladesh
Incubator 30 ºC	JSR, Model-JSGI 100T, Australia
Incubator 37 ºC	Friocell, England
Incubator 42 ˚C	Memmert, Germany
Glassware	Pyrex brand, USA.
Magnetic stirrer	LABTech co., LTD, Korea
Microcentrifuge, Eppendorf centrifuge	Germany.
Micropipettes	Eppendorf, Germany
Micropipette tips	Labsystems, Finland.
Microwave oven	Sharp R-380V(S), Thailand
PCR machine	MJ Research.
Power supply	BIO-RAD, USA.
pH meter	INOLAB WTW series
Stomacher Bag	Seaward, England
Stomacher machine	Seaward Stomacher 400 circulator